Review

Clinical review: A paradigm shift: the bidirectional effect of inflammation on bacterial growth. Clinical implications for patients with acute respiratory distress syndrome

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Abstract

Clinical studies have shown positive associations among sustained and intense inflammatory responses and the incidence of bacterial infections. We hypothesized that cytokines secreted by the host during acute respiratory distress syndrome may indeed favor the growth of bacteria and explain the association between exaggerated and protracted systemic inflammation and the frequent development of nosocomial infections. To test this hypothesis, we conducted *in vitro* studies evaluating the extracellular and intracellular growth response of three clinically relevant bacteria in response to graded concentrations of pro-inflammatory cytokines tumor necrosis factor-α, IL-1β, and IL-6. In these studies, we identified a U-shaped response of bacterial growth to pro-inflammatory cytokines. When the bacteria were exposed *in vitro* to a lower concentration of cytokines, extracellular and intracellular bacterial growth was not promoted and human monocytic cells were efficient in killing the ingested bacteria. Conversely, when bacteria were exposed to higher concentrations of pro-inflammatory cytokines, intracellular and extracellular bacterial growth was enhanced in a dose-dependent manner. The bidirectional effects of proinflammatory cytokines on bacterial growth may help to explain the frequent occurrence of nosocomial infections in patients with unresolving acute respiratory distress syndrome.

Keywords adult respiratory distress syndrome, bacteria, bacterial growth, infection, inflammation

The ability to generate and respond to signaling molecules establishes a mechanism for regulated cell-to-cell communication. Cells coordinate their growth and proliferation with autocrine and paracrine signaling by means of low molecular weight polypeptides called cytokines. Innate or natural immunity is a highly conserved defense mechanism against infections found in all multicellular organisms [1]. The inflammatory reaction is a fundamental component of the innate immune response, and its most proximal expression is characterized by the elaboration of proinflammatory cytokines – tumor necrosis factor (TNF)-α and interleukin (IL)-1β. Response to cytokines is generally viewed as exclusive to cells containing a defined nucleus, since cytokines are intended to work on well-defined eukaryotic cells with consequent signal transduction events.

When proinflammatory cytokines are present in optimal concentration, they recruit both specific and nonspecific immune cells, nonlymphoid leukocytes (monocytes/macrophages, neutrophils, basophils, and eosinophils), and lymphocytes to the site of assault and activate them, thereby helping to eradicate the assault and to restore homeostasis [2]. There are occasions, however, when the host defense response, in terms of inflammation, is exaggerated and protracted. In such cases, this primary defense process may instead cause enhanced tissue injury and maladaptive repair, leading to vital organ dysfunction and failure [3]. Reduction in the effective concentration of proinflammatory mediators is an important component in the resolution of inflammation [4].

ARDS = acute respiratory distress syndrome; BAL = bilateral bronchoalveolar lavage; CFU = colony-forming unit; IL = interleukin; LPS = lipopolysaccharide; NI = nosocomial infection; PMN = polymorphonuclear; TNF = tumor necrosis factor; VAP = ventilator-associated pneumonia.

The concepts of microbial etiology and pathogenesis of infectious diseases have undergone revisions since the pioneer researcher of microbial diseases, Robert Koch, postulated the criteria for microbial etiology of diseases. During the past decade, the emphasis in the study of the pathogenesis of infectious diseases has shifted from determining the function of the cellular players in the inflammatory response to the mediators that orchestrate this response [5]. The relationship between bacteria and inflammation is traditionally viewed as unidirectional. Bacteria trigger inflammation, which – as part of the host innate immune response – destroys bacteria and localizes the spread of infection. Although correct, this simple relationship does not provide a complete picture of the pathogen–host interaction in acute life-threatening infections. This unchallenged (preconditioned) view of the pathogen–host interaction has influenced for years the interpretation of objective clinical data in critical care medicine. In this paper, the clinical literature on nosocomial infections (NIs) in acute respiratory distress syndrome (ARDS) will be reviewed. The results will then be presented of a prospective study of ARDS patients that investigated longitudinally the relationship between circulatory and pulmonary proinflammatory cytokine levels, infections, and outcome. The findings of this study, as well as those of other groups, generated a novel hypothesis, suggesting that bacteria may grow in the presence of excessive cytokine levels. The results of recent *in vitro* studies from our group in support of this new hypothesis will also be reported.

Clinical observation in ARDS

ARDS is a frequent form of hypoxemic respiratory failure, characterized by the acute development of diffuse lung inflammation. In mortality data, after day three of ARDS, most patients die following a prolonged period of ventilatory support, during which they often develop fever and other criteria for systemic inflammatory response syndrome [6], clinical manifestations of infection [7–9], and multiple organ dysfunction syndrome [10,11]. In the medical literature, sepsis is associated with fatality in 36% to 90% of ARDS nonsurvivors [7,8,10,11]. At necropsy, 69% of ARDS nonsurvivors have histologic evidence of pneumonia [12]. These observations led to the hypothesis that, in ARDS, a direct correlation may exist between development of NIs, amplification of the systemic inflammatory response, and higher mortality [13]. Faist and coworkers [14] proposed a two-hit hypothesis in which NIs represent a second insult to a previously injured and primed host, converting a low-grade or regulated host response into an accelerated or dysregulated host response (accelerated systemic inflammatory response syndrome), triggering new or progressive organ dysfunction. Support for this hypothesis, however, relied only on clinical studies that did not use strict criteria for diagnosing NI. Furthermore, this broadly accepted pathophysiological hypothesis (second hit hypothesis) was never tested prospectively in ARDS.

Nosocomial infections and inflammation

Nosocomial infections and systemic inflammatory response in ARDS

We conducted a prospective study to investigate, at the onset of ARDS and during the progression of the disease, the longitudinal relationship between circulatory proinflammatory cytokine levels, infections, and outcome [15]. In most patients, the etiology of ARDS was pulmonary or extrapulmonary sepsis. We reported that, at the onset of ARDS, and over time, nonsurvivors ($n = 17$) had significantly ($P < 0.001$) higher plasma TNF-α, IL-1β, and IL-6 levels than survivors ($n = 17$) did [16]. During the first week of ARDS, plasma cytokine levels declined in all survivors, whereas they remained persistently elevated in all nonsurvivors. NIs were more frequent in patients with persistent cytokine elevation over time. The rate of nosocomial infection per day of mechanical ventilation was 1% in survivors and 8% in nonsurvivors. Moreover, none of the proven ($n = 36$) or suspected ($n = 55$) NIs caused either a transient or a sustained increase in plasma TNF-α, IL-1β, IL-6, and IL-8 levels above preinfection values [15]. This latter finding is in agreement with the recent understanding of downregulation (also called lipopolysaccharide [LPS] tolerance) of an activated system (see discussion in reference [15]). In these patients, a plasma IL-1β >400 pg/ml on day seven of ARDS was 100% accurate in predicting outcome [15]. Sixty-seven percent of NI developed after day 10 of ARDS, and among nonsurvivors, 15 out of 18 NIs developed while plasma IL-1β was >400 pg/ml. In addition to our work [15], one other study have described an association between high circulating IL-6 levels and increased rate of infections [17].

Ventilator-associated pneumonia and pulmonary inflammation in ARDS

The relationship between ventilator-associated pneumonia (VAP) and pulmonary inflammation was evaluated in a series of prospective studies. We evaluated with bilateral bronchoalveolar lavage (BAL) 94 ARDS patients with 172 episodes of suspected VAP and compared BAL results from contralateral sites [18]. Thirty-three of the 55 (60%) positive bronchoscopies had significant (>10^4 CFU/ml) growth in only one side. Episodes with bilateral significant growth were more likely to be polymicrobial, to have a bacterial growth >10^5 CFU/ml in the BAL, and to possess a higher percentage of polymorphonuclear (PMN) cells and intracellular microorganisms. These BAL findings indicated that episodes with a higher bacterial burden had cytological evidence of a more intense local inflammatory response and were more likely to be diffuse. Postmortem studies have also described a strong association between number of bacteria and severity of local inflammation [19–21]. The traditional interpretation of these data would suggest that the more severe inflammation was the result of a higher bacterial burden; however, this relationship was challenged by the results of our prospective study [22].

In a longitudinal study of patients with ARDS, subjected to bilateral BAL weekly and when clinical manifestations of VAP developed, we reported that at the onset of ARDS and over time, nonsurvivors had significantly ($P < 0.001$) higher BAL TNF-α, IL-1β, and IL-6 levels than survivors did [22]. Nonsurvivors had a higher rate of VAP than survivors [15]. In 21 episodes of VAP (16 unilateral and five bilateral pneumonia) there was excellent agreement between right and left BAL TNF-α, IL-1β, IL-6, total protein, and albumin levels. In other words, patients with unilateral pneumonia had similar TNF-α, IL-1β, and IL-6 levels in the BAL obtained from the lung with significant bacterial growth compared to the BAL from the contralateral lung without growth [15]. Furthermore, VAPs were not associated with either a transient or a sustained increase in BAL TNF-α, IL-1β, IL-6, and IL-8 levels above pre-infection values [15]. In agreement with our results, the findings of a recent experimental study of gram-negative pneumonia indicated that persistent elevation in BAL proinflammatory cytokines is associated with failure to clear intrapulmonary bacteria, despite a large influx of PMN in the airspaces [23].

Experimental and human studies have shown that a lung affected by ARDS is impaired in its ability to clear a bacterial challenge. Several intrinsic defects have been previously implicated, primarily those related to changes in the alveolar environment and the function of phagocytic cells [2]. Polymorphonuclear cells recruited into the airspaces of patients with ARDS have shown evidence of impaired microbicidal activity [24,25]; this mechanism partly explains the lung's inability to clear bacteria in spite of intense local inflammation. Furthermore, PMN clearing of bacteria is dose dependent, and the efficiency of PMN bactericidal activity decreases with increasing bacterial load [26].

Recent understanding of bacteria and cytokine interaction

In the interaction between a microorganism and its host, the host's defense does not go unchallenged [27]. Several reports have shown that DNA viruses have the ability to interfere with extracellular cytokines or inhibit cytokine synthesis [27]. Until recently, very little was known about the ability of bacteria to interfere with, or to utilize, extracellular cytokines secreted by the host cells or intracellular cytokines within phagocytic cells. Recent reports have shown that certain bacteria have receptors for cytokines IL-1β and TNF-α, and that exposure of bacteria to these cytokines enhanced their growth [28–30].

Receptors

The surfaces of bacteria have receptors for proinflammatory cytokines. Gram-negative bacteria have receptors for TNF-α and IL-1β [29–31], and the virulence property of the bacterium is altered as a consequence of cytokine binding [30]. Porat *et al.* [28] reported that virulent strains of *Escherichia coli* express receptors for IL-1β and demonstrated enhanced extracellular *in vitro* growth in the presence of biologically active recombinant IL-1β. Luo *et al*. [30] reported that TNF-α could bind efficiently to many strains of gram-negative bacteria and that TNF-α–bacterium complexes can interact with TNF-α receptors present on eukaryotic cells. They also showed that TNF-α binding enhanced bacterial invasion of HeLa cells and phagocytosis by human and murine macrophages [30]. We recently reported that the surfaces of *Staphylococcus aureus* have receptors for IL-1β [32].

Enhanced bacterial growth with cytokines

Enhanced bacterial growth in the presence of cytokines has been reported for *E. coli* (IL-1β [28], interferon-γ [33], IL-2 [34], and granulocyte macrophage colony stimulating factor [34]) and *S. aureus* (IL-4) [35]. Two studies have reported that the intracellular growth of *Mycobacterium avium*–intracellular complex was enhanced in human peripheral blood monocytes activated with the cytokines IL-3, IL-6, and granulocyte macrophage colony stimulating factor [36,37].

Anti-inflammatory cytokines have also been reported to promote bacterial growth. Two studies have shown that IL-10 and IL-4 can enhance the intracellular replication of bacteria. Park and Skerrett [38] reported that priming of human monocytes with IL-10 significantly enhanced the intracellular growth of *Legionella pneumophila*. Hultgren *et al.* [35] reported reduced growth of *S. aureus* in the joints of an IL-4-deficient mouse and showed that exposure of macrophages to IL-4 reduced intracellular killing of *S. aureus* without impairing phagocytosis. We recently reported that the extracellular growth of *S. aureus* is enhanced in the presence of IL-1 receptor antagonist [32].

New hypothesis and hypothesis testing

The findings from our studies described above [15,16,22] suggested that final outcome in patients with ARDS is related to the magnitude and duration of the host inflammatory response, and that intercurrent NIs might be an epiphenomenon of prolonged intense inflammation. The increased rate of NIs might be explained by impaired host defense response. We hypothesized, however, that cytokines secreted by the host during ARDS may indeed favor the growth of bacteria and explain the association between an exaggerated and protracted release of cytokines and the frequent development of NIs.

To test this hypothesis, we conducted *in vitro* studies evaluating the extracellular and intracellular growth response of three clinically relevant bacteria in response to graded concentrations of proinflammatory cytokines TNF-α, IL-1β, and IL-6 [32,39–41]. The bacteria used were fresh isolates of *S. aureus*, *Pseudomonas aeruginosa,* and *Acinetobacter* sps obtained from patients with ARDS. The bacteria were grown in 3 ml of RPMI/DMEM medium without serum or antibiotics. Intracellular growth was tested in the human monocytic cell line, U937, and in blood monocytes of normal healthy volunteers.

In these studies, we identified a U-shaped response of bacterial growth to proinflammatory cytokines. When the bacteria were exposed *in vitro* to a lower concentration (10–250 pg) of TNF-α, IL-1β, or IL-6 – similar to the plasma values detected in ARDS survivors [16] – extracellular and intracellular bacterial growth was not promoted, and human monocytic cells were efficient in killing the ingested bacteria [39,40]. Conversely, when bacteria were exposed to higher concentrations of these of proinflammatory cytokines – similar to the plasma values detected in ARDS nonsurvivors [16] – intracellular and extracellular bacterial growth were enhanced in a dose-dependent manner [39,40]. Blockade by specific neutralizing monoclonal antibodies significantly inhibited cytokine-induced extracellular and intracelullar bacterial growth [39,40].

The effects of cytokines on extracellular bacterial growth were seen only with fresh isolates and were lost after six *in vitro* passages [39]. These findings indicate that, in the host milieu, *S. aureus*, *Ps. aeruginosa*, and *Acinetobacter* sps may acquire a phenotypic ability to use cytokines as growth factors; subsequent removal of these pathogens from such milieu (after six *in vitro* passages) resulted in the loss of the acquired phenotype. This phenomenon of loss of responsiveness to cytokines was also recorded by Porat and collaborators [31].

Effects of LPS on intracellular bacterial growth

The intracellular growth of *S. aureus*, *Ps. aeruginosa,* and *Acinetobacter* sps was also tested after exposure of U937 monocytic cells to graded concentrations of LPS. At low priming concentrations of LPS, we observed a significant reduction in intracellular bacterial growth compared to the control. At a priming concentration of LPS equal to, or greater than, 100 ng, however, all three bacterial isolates had a significant growth enhancement compared to the control ($P < 0.0001$, for all three bacteria). Taken together, our findings indicate that there may be a threshold of cellular activation at which phagocytic cells effectively kill ingested bacteria. Above this threshold of cellular activation, however, the intracellular micromilieu becomes favorable to the survival and replication of the ingested bacteria. It is likely that bacteria that are internalized, and under selective pressure, may adapt to an otherwise hostile microenvironment by switching on novel gene expression that enables them to utilize cytokines as their growth factors.

Effects of methylprednisolone on intracellular bacterial growth

We exposed U937 monocytic cells primed with the highest concentration of LPS (10 μg) to escalating concentrations (0 μg, 25 μg, 50 μg, 75 μg, 100 μg, 150 μg, and 250 μg) of methylprednisolone and quantified both intracellular bacterial growth and the intracellular transcription of TNF-α, IL-1β, and IL-6. We found that exposure of LPS-primed U937 monocytic cells to methylprednisolone prior to infection affected (in a dose-dependent manner) the mRNA expression of TNF-α, IL-1β, and IL-6, and the *in vitro* intracellular bacterial growth of internalized *S. aureus*, *Ps. aeruginosa,* and *Acinetobacter* sps [41].

The impairment in intracellular bacterial killing correlated with the increased expression of proinflammatory cytokines, while restoration of monocyte killing function upon exposure to methylprednisolone coincided with the downregulation of the expression of TNF-α, IL-1β, and IL-6. We found that, at the two highest concentrations of methylprednisolone (150 μg and 250 μg), the mRNA expression of all three cytokines was significantly blunted, irrespective of the LPS concentration. Hence, we presume that bacterial survival and replication within the phagocytic cells are functions of the cytokines expressed by such cells. In the presence of excessive activation, the intracellular environment appears to favor the emergence of new phenotypes of bacteria that are capable of utilizing cytokines for their growth. By showing that methylprednisolone can reduce (in a dose-dependent manner) the mRNA expression of TNF-α, IL-1β, and IL-6, and the intracellular bacterial growth of the bacteria, we provide experimental evidence to suggest a cause-and-effect relationship between excessive inflammation and bacterial growth.

Bacteria and cytokine interactions

It is unclear how bacteria may use cytokines for their growth, since bacteria are prokaryotes without a defined nucleus and cytokines are intended to work on well-defined eukaryotic cells with consequent signal transduction events. In a host milieu, however, bacteria may adapt to eukaryotic cellular processes [42]. Although, the subsequent sequence of intracellular events has not been delineated, it is possible that bacteria might use cytokines through receptor-mediated, signal-transduction-induced activities that would require the presence of biochemical processes akin to those seen in eukaryotic cells. Cytokines may act on bacteria through a signaling process similar to that of eukaryotes, but involving different biochemical pathways, or bacteria may break down cytokines into biologically active fragments that are transported across the bacterial cell membranes and act on specific gene transcription and translation.

To further elucidate the complex interactions between bacteria and cytokines, we conducted *in vitro* experiments using *S. aureus* to demonstrate the presence of IL-1 receptors on the surface of *S. aureus*, and to localize the region(s) of IL-1β that enhance the extracellular growth of *S. aureus*. We identified an IL-1β receptor on the surface of the bacterium and we utilized five linear peptide fragments of human IL-1β and whole biologically active molecules of both IL-1 receptor antagonist and IL-1β to study their effects on extracellular growth of *S. aureus*. Of the five peptide fragments studied, the 208–240 peptide fragment demonstrated the most pronounced effect on the growth of *S. aureus.* Previously, this fragment has been shown to be pyrogenic and to enhance

Table 1

Traditional versus alternative interpretation of clinical data on nosocomial infections and inflammation in ARDS

	Traditional interpretation	Alternative interpretation
Inflammation and bacteria	Inflammation kills bacteria	Regulated inflammation kills bacteria while excessive (unregulated) inflammation may enhance bacterial growth
Nosocomial infections	More frequent in nonsurvivors	More frequent in patients with persistent cytokine elevation
	Amplify inflammation (second hit hypothesis) and worsen multiple organ dysfunction syndrome	Do not amplify inflammation (downregulation, or LPS tolerance)
Systemic inflammation in ARDS	Progression is amplified by nosocomial infections (≥day 3 of ARDS)	Progression is determined prior to day 3, by the success and/or failure of the host regulatory mechanisms
Glucocorticoid treatment in patients with unregulated systemic inflammation	Causes immunosuppression and enhances the risk for developing infections	If given in low doses for a prolonged period (≥7 days) may have an important immunomodulatory effect in regulating excessive inflammation and restoring homeostasis

ARDS, acute respiratory distress syndrome; LPS, lipopolysaccharide.

sleep in rabbits [43]. This peptide fragment, however, does not have mitogenic activity on T cells *in vitro*, indicating its probable lack of ability to interact specifically with IL-1 receptor (although the possibility of a receptor–ligand interaction cannot be completely ruled out). This peptide (208–240) enhanced the growth of *S. aureus* approximately 22-fold compared to the control (bovine serum albumin). Another peptide spanning amino acids 118–147 also enhanced the extracellular growth of *S. aureus* significantly, although much less efficiently, than the peptide fragment 208–240. No significant effects were exerted on *S. aureus* growth by the other 3 fragments studied. No biological activities in any other systems have been reported for any of the peptides studied other than for the one spanning 208–240.

Several proteinases are released from *S. aureus* into the extracellular medium [44] and it is therefore possible that such enzymes may cleave the IL-1β molecule into peptide fragments. Such short peptide fragments may then be transported across the bacterial cell membrane and act as direct growth factors, or as transcription factors, for the production of bacterial growth factors. This remains speculative at this time since there is no direct proof of such cleavage activities of *S. aureus* extracellular proteinases on IL-1β. There are, however, reports of proteolytic cleavages of IL-2, TNF-α, and/or interferon-γ by bacterial products of *Ps. aeruginosa* and *Legionella pneumophila* [5].

Conclusion

The bidirectional effects of proinflammatory cytokines on bacterial growth may help explain the frequent occurrence of NIs in patients with unresolving ARDS. Table 1 shows the traditional versus alternative interpretations of clinical findings in ARDS. If NIs in unresolving ARDS are indeed an epiphenomenon of exaggerated inflammation, it follows that treatment modalities that effectively decrease cytokine synthesis may reduce the incidence or severity of NIs.

Competing interests

None declared.

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