Review

Bench-to-bedside review: Biotrauma and modulation of the innate immune response

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Abstract

The innate immune network is responsible for coordinating the initial defense against potentially noxious stimuli. This complex system includes anatomical, physical and chemical barriers, effector cells and circulating molecules that direct component and system interactions. Besides the direct effects of breaching pulmonary protective barriers, cyclic stretch generated during mechanical ventilation (MV) has been implicated in the modulation of the innate immunity. Evidence from recent human trials suggests that controlling MV-forces may significantly impact outcome in acute respiratory distress syndrome. In this paper, we explore the pertinent evidence implicating biotrauma caused by cyclic MV and its effect on innate immune responses.

Introduction

The natural or innate immune system is present in some form in most living organisms and consists of mechanisms for defending the host against foreign invaders and for healing injured tissues. We now know that many of the mechanisms of resistance to infection are also involved in the individual's response to noninfectious foreign substances and environmental stresses, including mechanical stretch. Furthermore, mechanisms that normally protect individuals and eliminate foreign substances are themselves capable of causing tissue injury and disease. This inherent defense network includes anatomical, physical and chemical barriers, circulating molecules, cells with specific phagocytic or lytic abilities, and soluble mediators that orchestrate the activities of each component and their interactions with the acquired immune system. Normally, this is a well integrated system of host defense and preservation of self-integrity, in which numerous cells and molecules function cooperatively. However, dysregulation of the fine balance between proinflammatory and anti-inflammatory stimuli may explain the pathophysiologic processes that underlie syndromes such as sepsis and acute lung injury (ALI) [1].

Although patients undergoing positive pressure mechanical ventilation may have impaired lung function, and possibly impaired systemic immune defenses by virtue of their underlying lung pathology, further dysregulation of natural defenses occurs in these patients. The presence of an endotracheal tube bypassing natural upper airway defenses, decrease or loss of coughing, paralysis of bronchial ciliae, alterations in surfactant and phagocyte and epithelial defensins – a critical first line antibacterial defense mechanism – all contribute to impairment in host defense [2–4]. Apart from the direct effects of breaching pulmonary protective barriers, cyclic stretch generated during mechanical ventilation has been implicated in the modulation of the innate immune system. In this short review we revisit some of the pertinent evidence exploring the relationship between biotrauma caused by cyclic mechanical ventilation and its effect on innate immune responses. This is not intended to be a comprehensive and structured review of the

ALI = acute lung injury; ARDS = acute respiratory distress syndrome; CXCR = CXC chemokine receptor; IL = interleukin; LPS = lipopolysaccharide; MIP = macrophage inflammatory protein; MODS = multiple organ dysfunction syndrome; PEEP = positive end-expiratory pressure; PIP = peak inspiratory pressure; PMN = polymorphonuclear neutrophil; Th = T-helper (cell); TNF = tumor necrosis factor; VILI = ventilator-induced lung injury; Vt = tidal volume.

topic, but a window into what is novel in the basic science field of ventilator-induced lung injury (VILI) and what challenges there are for the future.

Biotrauma and multiorgan failure

Patients with acute respiratory distress syndrome (ARDS) have a serious form of ALI with a mortality rate of at least 30% [5–8]. However, the vast majority of patients who die with ARDS do not die from their pulmonary disease (hypoxia) but rather from dysfunction of other organs, termed multiple organ dysfunction syndrome (MODS) [9,10]. A number of animal and clinical studies have shown that mechanical ventilation *per se* can worsen pre-existing lung injury and produce VILI. This topic has been the subject of a number of reviews [9–14]. The spectrum of VILI includes not only air leaks and increases in endothelial and epithelial permeability, but also increases in pulmonary and systemic inflammatory mediators – a process that has been termed 'biotrauma' [9,10].

Overdistension and shear stress forces generated during some patterns of mechanical ventilation have been implicated in the pathophysiology of the inflammatory response associated with VILI [15–19]. Alterations in the levels of various proinflammatory and anti-inflammatory mediators secondary to mechanical injury may play a crucial role in potentiating and/or propagating this systemic inflammatory reaction, ultimately leading to MODS and death. The central concept is that mediators originate in the lung and gain access to the circulation where they potentially can exert detrimental effects. There are several principal mechanisms by which mediator release may occur after cyclic stretch: stress failure of the alveolar epithelial–endothelial barrier (decompartmentalization); stress failure of the plasma membrane (necrosis); alterations in cytoskeletal structure without ultrastructural damage (mechanotransduction); and effects on vasculature independent of stretch or rupture. Irrespective of the precise mechanism(s) of mediator release, the clinical consequences may be devastating. The cumulative evidence that implicates VILI as a direct causative agent for MODS was recently reviewed [14,16].

'Injurious' mechanical ventilations strategies – large tidal volume (Vt; usually >12 ml/kg) and zero positive end-expiratory pressure (PEEP) in experimental conditions – in previously injured lungs can promote the release of inflammatory mediators in the lungs and worsen lung injury. This is supported by evidence from *in vitro* cell-stretch systems, from *ex vivo* lung models, and from *in vivo* models of mechanical ventilation following lung lavage, aspiration, or endotoxin administration [20–24]. Damage to normal (noninjured) or injured lungs by the application of very high Vt (30–40 ml/kg) or very high inspiratory pressures has also been documented. (Detailed discussions of the possible mechanisms of VILI are provided elsewhere [11,12,15,25].) Clinical studies have also provided convincing evidence that high Vt ventilation can lead to an increase in production of inflammatory mediators in humans [26–28]. The clinical significance of VILI became apparent after the ARDSNet demonstrated that a 'lung protective approach' – lowering Vt to 6 ml/kg (predicted body weight) – was associated with increased survival in ARDS patients [28]. One of the possible explanations for this is that ventilatory strategies that limit overdistension attenuate the effects of biotrauma. Support for this theory can be inferred from the decrease in plasma IL-6 levels in patients who were ventilated with the protective strategy. Presumably, the lower IL-6 level in these patients reflects a reduction in the proinflammatory response secondary to decreased biotrauma to the lung. Ranieri and coworkers further expanded on this hypothesis by demonstrating that what was previously thought of as a conventional ventilation strategy (12 ml/kg) can lead to an increase in both local and systemic inflammatory mediators [27], and that an increase in plasma IL-6 levels correlates with the development of MODS [27,29].

Although there is no direct evidence to date that definitively demonstrates that mediators generated in the lung can cause MODS, injurious ventilatory strategies can lead to release of a number of factors that could theoretically have an impact on MODS, including translocation of bacteria, bacterial products, or circulating proapoptotic factors [30–33]. In support of the link between VILI and MODS, Imai and coworkers [33] demonstrated that an injurious ventilation strategy in animals with lung injury due to acid aspiration led to apoptosis in the kidneys and small intestine. The authors also found a significant correlation between changes in soluble Fas ligand (a key mediator of cellular apoptosis) levels and changes in creatinine in patients with ARDS involved in a clinical trial of protective ventilation strategy. Further evidence is required to determine whether the soluble Fas ligand actually originated in the lungs. Irrespective of the source, these findings may have important biologic and clinical implications.

VILI can modulate polymorphonuclear neutrophil function and innate immune response to lipopolysaccharide and sepsis

The general strategy of innate immune detection is one in which a limited number of receptors are dedicated to the recognition of microbial molecules that are conserved across broad taxa, and, for the most part, the receptors must be indifferent to molecules of host origin (the basis of innate immune discrimination between self and non-self) [34]. Recently, it has become apparent that the term 'pathogen-associated microbial pattern' is a misnomer. In fact, it is not microbial patterns that are recognized but rather specific molecules, that are integral constituents of microorganisms that are recognized, suggesting this system is highly discriminatory [35,36]. Moreover, it is now evident that the innate immune response can be be altered, enhanced or suprressed. In small doses, lipopolysaccharide (LPS; a primary component of Gram-negative bacteria) can render

animals resistant to a subsequent pathogen challenge. LPS has a strong adjuvant effect, and it is well known that certain microbes enhance the response to a co-injected protein antigen [37,38]. This is of primary importance to critical care physicians because there is a growing body of evidence in support of the theory that mechanical ventilation may sensitize the innate immune system and that, in turn, the innate immune system may sensitize the lungs to the effects of mechanical ventilation. This 'two-hit hypothesis' has permeated the literature on VILI and purported ensuing MODS.

Pressure cycled ventilation can cause human alveolar macrophages to release cytokines and proteases *in vitro*, and the effect is amplified by bacterial LPS [39,40]. The ability of cyclic stretch to modulate specific immune function is not restricted to cells of myeloid origin [23]. In both alveolar epithelial cells and bronchial epithelial cells, cyclic stretch leads to increased expression of IL-8 [22,41]. Augmentation of this response is seen with co-stimulation with tumor necrosis factor (TNF)-α [42,43]. However, although the initial inciting event (mechanical ventilation) may be injurious, interaction between the innate immune system and mechanical injury may be required for the development of the full-blown lung injury phenotype of VILI.

Using a rat model of cecal ligation perforation, Herrera and coworkers [44] found that animals ventilated with high Vt (20 ml/kg for 3 hours) developed worse lung damage, higher cytokine synthesis and release, and higher mortality rates. Moreover, stabilizing alveoli in septic animals with PEEP (presumably reducing atelectrauma) resulted in attenuation of lung injury and reduced systemic and local inflammatory response as measured by levels of inflammatory mediators, and prevented animals from dying at a given time. Altemeier and coworkers [45] postulated that mechanical ventilation with moderately high Vt (15 ml/kg) can augment the inflammatory response in uninjured lungs to systemic LPS treatment, independent of biotrauma. In a rabbit model of ALI, those investigators found that mechanical ventilation alone resulted in minimal cytokine expression in the lung but it did significantly enhance LPS-induced expression of TNF-α, IL-8, and monocyte chomotactic protein-1. Two other important factors are worthy of mention in this study: systemic LPS was given in a modest dose (5 mg/kg) and did not result in overt ALI before initiation of the ventilation protocol; and the mechanical ventilation protocol used levels of Vt that did not lead to disruption of the epithelial cell membrane, as demonstrated by preservation of barrier function and absence of histologic changes consistent with structural disruption. Based on these findings, the authors postulated that cyclic stretch interacts with innate immune components, which allows leakage of bacterial products, resulting in an enhanced inflammatory response. One potential interaction is with endotoxin; another potential mechanism is through activation of effector cells via the effects of cyclic stretch [46].

Polymorphonuclear neutrophils (PMNs) are among the most important effector cells of the innate immune system. Because of the consistent association between PMNs and lung injury in humans and experimental models, PMNs have been implicated as causative agents of both ALI and VILI. In rodent models of VILI, neutrophil migration into the alveoli appears to be in large part dependent on stretch-induced macrophage inflammatory protein (MIP)-2 production from both circulating and resident parenchymal cells [47]. Cyclic overstretching of normal rabbit lungs with large Vt (20 ml/kg) is known to produce neutrophil influx and an increase in IL-8 levels in bronchoalveolar lavage fluid [48]. Neutrophil depletion (vinblastine injection) has been shown to attenuate IL-8 increase in the lung. P-selectin or intercellular adhesion molecule-1 (key cell membrane proteins that are involved in endothelial cell activation) are not expressed in animals depleted of their neutrophils. These findings suggest that production of pulmonary IL-8 by lung overstretch might require interaction between resident lung cells and migrated neutrophils.

Activation of PMNs in VILI occurs primarily in the alveolar space after migration [49]. In a recent study, Belperio and coworkers [50] demonstrated that the stress generated by mechanical forces can lead not only to PMN accumulation but also to consequent PMN-induced changes in microvascular permeability in the lung. The ability of neutrophils to cause lung damage was mediated by increased expression of CXC chemokine receptor (CXCR)2 ligand in lung tissues (resident parenchymal cells) interacting with CXCR2 receptor on PMNs after mechanical injury. Blocking the CXCR2 receptor or CXCR2 ligand deficiency conferred protection against the deleterious effects of VILI.

Steinberg and coworkers [51] employed *in vivo* video microscopy to assess alveolar stability directly in normal and surfactant-deactivated lung. They showed that that alveolar instability caused mechanical injury and initiated an inflammatory response that resulted in a secondary neutrophil-mediated proteolytic injury. These findings suggest that PMNs can transmigrate into the lung without accompanying capillary damage, and that once in the alveolar space they become activated so that damage occurs in the lung.

Su and coworkers [52] recently found that initiation of low Vt ventilation (6 ml/kg body weight; PEEP 10 cmH_2O and fractional inspired oxygen 0.5) early in the course of a sheep model of polymicrobial septic shock prolonged the time to development of hypotension and anuria, and prolonged survival as compared with that in animals ventilated with a Vt of 12 ml/kg. The clinical implication is that use of prophylactic low Vt ventilation may obviate negative interactions between forces generated by the mechanical ventilator that affect the innate immune response, thus improving clinical outcome.

VILI can modulate the innate immune response to bacteria

Overinflation in certain models of mechanical ventilation has also been implicated in promoting translocation of bacteria [30,31] or bacterial products [32] from the lung into the circulation. Recent data indicate that mechanical ventilation may also predispose individuals to local (pulmonary) dissemination of bacteria and infection. Schortgen and coworkers [53] evaluated the effect of Vt reduction and alveolar recruitment on systemic and contralateral dissemination of bacteria and inflammation during right-sided pneumonia. One day after instillation of *Pseudomonas aeruginosa* into the right lung, rats were either left unventilated or ventilated for 2 hours using different ventilatory and alveolar recruitment strategies: low Vt (6 ml/kg) with either (a) no PEEP; (b) PEEP at 8 cmH_2O (c) PEEP at 8 cmH_2O in the left lateral decubitus position; (d) PEEP at 3 cmH_2O with partial liquid ventilation; or (e) high Vt to achieve end-inspiratory pressure of 30 cmH_2O without PEEP. All mechanical ventilation strategies with the exception of the low PEEP strategy promoted contralateral lung bacterial dissemination. Overall bacterial dissemination, as assessed by the number of positive splenic cultures, was lower in the nonventilated controls (22%) and low Vt/low PEEP (22%) group than in the high Vt/zero PEEP (67%) group. The mechanism by which increased local and systemic bacteremia occurs remains to be elucidated. The current leading hypothesis is that this is related to the process of translocation. Another possibility is that mechanical ventilation, by virtue of its effects on cytokine release (biotrauma), may alter bacterial growth patterns [54,55].

Mechanical ventilation not only may enhance the local and systemic dissemination, and perhaps growth of pathogenic bacteria, but it may also increase susceptibility to development of systemic bacteremia. In a recent study, Lin and coworkers [56] ventilated animals for 1 hour with either a protective strategy (Vt 7 ml/kg, PEEP 5 cmH_2O) or an injurious ventilatory strategy (Vt 21 ml/kg, zero PEEP). *P aeruginosa* was subsequently instilled intratracheally before extubation and animals were followed for 48 hours (breathing spontaneously). The mortality rate was 28% in the protective ventilation group and 40% in the injurious ventilation group. In that study, a protective ventilation strategy was associated with lower incidence of positive bacterial cultures in the lung ($P = 0.059$) and in the blood ($P < 0.05$). Note that the significance of the strategy chosen in this study was that bacterial instillation occurred after completion of the mechanical ventilation protocol, presumably when ongoing injury to the capillo–alveolar membrane was no longer taking place. In this context, mechanical ventilation with high Vt and zero PEEP would somehow sensitize the lung to systemic bacteremia. Concentrations of blood TNF-α and MIP-2 were also significantly higher in the low Vt groups than in the high Vt group, suggesting that innate immune responses may be tailored to specific compartments.

VILI and systemic immunosuppression: what impact do this have on the biotrauma hypothesis?

The general consensus is that cyclic stretch may lead to upregulation of inflammatory/immune/injurious responses in the lung. Recent evidence suggests that the systemic consequences of cyclic stretch may be immunosuppression. Vreugdenhil and coworkers [57] recently explored the role played by different ventilatory strategies on peripheral immune cell function in healthy rats. Normal rats were ventilated for 4 hours with one of the following strategies: low peak inspiratory pressure (PIP; 14 cmH_2O)/PEEP; high PIP (32 cmH_2O)/PEEP; and high PIP/zero PEEP. In these experiments peripheral natural killer cell activity, mitogen-induced splenocyte proliferation, and chemokine/cytokine production (MIP-2 and IL-10) decreased after high PIP/PEEP ventilation. Interferon-γ production was also significantly lower than in the low PIP/PEEP group. Plotz and coworkers [58] noted remarkable changes in the immune response of infants without pre-existing lung pathology who were being ventilated during cardiac procedures. In the lungs (locally), the immune balance favored a proinflammatory response pattern without detectable concentrations of anti-inflammatory mediators. In the systemic circulation, the functional capacity of peripheral blood leukocytes to produce interferon-γ, TNF-α, and IL-6 *in vitro* was significantly decreased. This was accompanied by a significant decrease in the killing activity of natural killer cells. These data support the theory that high positive inspiratory pressure ventilation leads to upregulation of local pulmonary response. Simultaneously, the peripheral immune response was downregulated.

The finding that mechanical ventilation can lead to systemic immunosuppression or immunodepression is controversial in that most other studies have found increases in systemic TNF-α as well as IL-6 and MIP-2 (rodent chemokine orthologous to IL-8) release following mechanical ventilation [27,28,59–61]. At this stage determining the cause of systemic immunosuppression is highly speculative. It is possible that both observations are true. The state of systemic immunosuppression could precede the acute rise in proinflammatory mediators. In recent years considerable evidence has accumulated suggesting that 'injurious' mechanical ventilation strategies, particularly when applied to injured lungs, causes the release of inflammatory mediators, which may then pass on to the circulation [9,21,24,27]. The main theory in support for increasing levels of inflammatory mediators in the serum in ARDS is loss of pulmonary compartmentalization; in VILI, loss of capillary–alveolar membrane integrity presumably occurs due to mechanical injury and biotrauma. However, in the absence of gross loss of membrane integrity, it is possible that systemic release of inflammatory mediators may not occur. This would explain the absence of systemic immune system mediators but not the presence of systemic immunosuppression.

Munford and Pugin [62] hypothesized that local inflammation is often accompanied by systemic anti-inflammatory responses. The teleologic advantage of coordinating local inflammation with systemic anti-inflammation is that it may allow for the immune system to focus its efforts on containing the local inflammation while preventing potentially injurious inflammation in unaffected sites. This 'immuno-paralysis' has been felt to be a consequence of unbalancing proinflammatory and anti-inflammatory responses. Another equilibrium-related hypothesis relates to altered Th1/Th2/Th3 balance in the periphery, with subsequent preponderance of a Th2/Th3 response that disturbs the balance of T-effector cells in the periphery. An alternative explanation relies on the activation of the adrenergic nervous system. Catecholamine secretion is activated by physical stress leading to activation of the β_2 receptors on cells of both myeloid and nonmyeloid origin, resulting in the downregulation of proinflammatory cytokines and upregulation of anti-inflammatory mediators such as IL-10 and transforming growth factor-β [14]. Again, an imbalance in this response may result in significant peripheral immunosuppression [14].

The main criticism of these theories is that they would presumably not be exclusive to the experimental models mentioned above, and would hence affect any model of ALI. The unique features of the two studies that detected systemic immunosuppression relate to the fact that in both cases mechanical ventilation was not a particularly injurious protocol and was applied to normal lungs (previously uninjured lungs). Herein may lie the explanation for these intriguing findings; in the absence of a potent innate immune activation signal, either locally or systemically (LPS, TNF-α, bacteria, severe damage to the capillo–alveolar membrane, or other), systemic immune suppression may be the response to mechanical ventilation-induced lung injury (by virtue of any of the balance hypotheses or a combination of different hypotheses). This may not have been detected previously because very few studies addressed systemic immune function after mechanical ventilation of normal lungs; in fact, in the only other study looking at sytemic inflammatory mediators after mechanical ventilation in normal adult lungs, no change in the systemic pro-inflammatory or anti-inflammatory profile was noted [63]. Under this hypothesis, the effects of mechanical ventilation would be entirely dependent on the environmental milieu. A recent study conducted by Gurkan and coworkers [64] suggested that compartmental regulation of gene expression occurs in association with differential ventilation strategies in distal organs. In that study, the expression of vascular endothelial growth factor decreased in the liver but increased in the kidney in response to different ventilation strategies. Moreover, pulmonary repair mechanisms are likely to play an active role in determining the ultimate outcome of local injury and ensuing systemic derangement.

Conclusion

The clinical importance of appreciating the role played by innate immunity in VILI goes beyond understanding what we do to patient's immune systems when we initiate the life-saving procedure of mechanical ventilation. The observations underscoring the potentially critical relationships between mechanical ventilation, inflammation, infection, and innate immunity provide a rationale for interrupting or modifying innate immune pathways in the lungs in patients at risk for lung injury or at the onset of lung injury. The good news for intensivists is that, unlike other problems that we deal with in the intensive care unit, we know exactly when VILI begins – with the initiation of mechanical ventilation. Consequently, immune therapy may be a feasible option in the future to prevent or reduce VILI.

Competing interests

The author(s) declare that they have no competing interests.

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Correction: The effect of activated protein C on experimental acute necrotizing pancreatitis

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After publication of this work [1] we noticed the following errors: The surname of the first author was incorrectly written as 'Yamenel' and should be 'Yamanel.' In the Study Protocol section of the materials and methods, the units for APC dosage should be 'µg/kg' not 'mg/kg.' Please see the corrected section below. There is a spelling mistake in the fourth paragraph of the discussion. 'Refect' should read 'reflect.'

Study Protocol

After the stabilization period, 45 male rats were randomly divided into three groups. Rats in group I (control group; $n = 15$) underwent laparotomy with manipulation of the pancreas (sham procedure) and received 10 ml/kg saline intravenously (single dose). Groups II and III underwent laparotomy with induction of ANP. Rats in group II (positive control; $n = 15$) received saline, as in group I but 6 hours after induction of ANP. Rats in group III (treatment group; $n = 15$) received 100 µg/kg recombinant human APC (Drotrecogin alfa [activated]; Xigris; Lilly, Istanbul, Turkey) intravenously (single dose) 6 hours after induction of ANP. Twenty-four hours after induction of ANP, all surviving animals were killed by intracardiac infection of pentobarbital (200 mg/kg). Blood samples were taken from the heart before the animals were killed in order to measure serum amylase, TNF-α, and IL-6. Animals that died before the end of the study (four in group II and two in group III) were excluded from the analysis.

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