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Mechanotransduction, ventilator-induced lung injury and multiple organ dysfunction syndrome

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Introduction

Ventilator-induced lung injury remains a significant problem in the care of critically ill patients [1]. A large body of data supports the concept that ventilation *per se* can be responsible for causing lung injury [1, 2]. The postulate is that structural disruption due to either overdistension or to shear forces generated during repetitive opening and collapse of atelectatic regions of the lung may exacerbate, or initiate, significant inflammation and lung injury [1, 2, 3]. Recently, a new mechanism of injury – termed biotrauma – has been elaborated in which the mechanical stresses produced by mechanical ventilation lead to the up-regulation of an inflammatory response, as evidenced by neutrophil infiltration in the lungs and increased broncho-alveolar lavage (BAL) levels of a host of inflammatory mediators [3, 4, 5]. Activation and/or propagation of the inflammatory cascade plays a pivotal role in the clinical outcomes of patients with acute lung injury (ALI) /acute respiratory distress syndrome (ARDS) [6].

Despite advances in critical care, the mortality in ARDS remains about 40–50 % and most patients who die, do so from multiple organ system failure (MOSF) rather than from hypoxia [7]. Recently investigators have explored the hypothesis that mechanical ventilation may play a pivotal role in the development of MSOF in certain patients, by causing an increase in inflammatory mediators in the circulation [4]; these mediators have been shown to play a critical role in the pathophysiology of MSOF and shock [8, 9].

The evidence that mechanical ventilation can initiate or exacerbate an inflammatory response comes mostly from animal data: (1) pathological evidence of neutrophil infiltration [10, 11], (2) increased cytokine levels in lung lavage [5] and (3) increased cytokine levels in the systemic circulation [12, 13]. Moreover, ventilatory strategies that were designed to minimize ventilator-induced lung injury in these animal studies were associated with significantly lower cytokine levels [5, 10]. How mechanical ventilation induces its deleterious effect is as of yet unclear. Studies in vitro and in vivo have found that both the degree and pattern of stretch are important in determining cellular response [5]. Lung stretch is known to be an important factor in lung growth and development, as well as surfactant production [13, 14]. Hence the postulate is that, by altering both the pattern and magnitude of lung stretch, mechanical ventilation may lead to alterations of gene expression or cellular metabolism. Among the proposed mechanisms by which cellular deformation is converted into changes in cell phenotype or metabolism are (a) direct conformational change in membrane-associated molecules leading to activation of downstream messenger systems, (b) activation or inactivation of stretch sensitive channels and (c) release of paracrine or autocrine factors.

Mechanotransduction is the conversion of mechanical stimuli, such as cell deformation, into biochemical and biomolecular cellular alteration [15]. However, the conversion of a mechanical stimulus to a biomolecular signal is presumably the first step in a sequential cascade of events which herald the systemic inflammatory response. In this paper we review four publications which have recently contributed to our understanding of the signal pathways leading from receptor activation to gen-

eration of second messengers which are able to mediate and orchestrate the elaborate inflammatory reaction observed in patients with ALI/ARDS and MOSF.

The nature of the primary cell mechanotransduction event responsible for converting an externally acting mechanical stressor into an intracellular signal cascade remains unclear. The first paper reviewed reports a novel, previously undescribed mechanism, by which physical forces induce expression of important regulatory genes. The second paper describes the utilization of magnetic twisting cytometry to document changes in cellular stiffness in human airway smooth muscle cells – the hypothesis being that modulating actin-myosin interaction would induce changes in cytoskeletal stiffness – which mimic those documented in acutely injured lungs. The third papers describes a clever artificial lung system that allows cells grown in monolayers to be exposed to both pressure and stretch/strain resembling that of conventional ventilation. Finally, the fourth paper consolidates the basic science data which suggests that mechanical injury to lung alveoli, as a consequence of mechanical ventilation, may in itself induce and/or exacerbate ALI/ARDS via the generation and promotion of a local, and then systemic, inflammatory response. Ranieri et al. demonstrated that mechanical ventilation per se may be an important factor in determining the pulmonary and systemic cytokine levels in patients with ARDS. In addition, they demonstrated that cytokine response may be attenuated by strategies that minimize overdistension and recruitment/derecruitment of the lung.

Grembowicz KP, Sprague D, McNeil PL (1999) Temporary disruption of the plasma membrane is required for c-fos expression in response to mechanical stress. Mol Biol Cell 10: 1247–1257

Endothelial fibroblasts and smooth muscle cells were injured by slowly scratching cell monolayers with a sterile needle. Fos mRNA and protein content in injured cell monolayers were analyzed. The importance of c-fos resides in the fact that it is a representative immediate-early response gene that contains a stretch responsive promoter [5]. Using this technique, this group was able to demonstrate that fos protein synthesis was increased in those cells lining the denudation tracts after plasma membrane disruption (PMD).

To demonstrate that PMD is an absolute requirement for fos response in this system, the protein synthesis inhibitor gelonin was added to injured cells. Injury in the presence of gelonin completely inhibited fos response. In addition, fos response was absent in the presence of supranormal calcium concentrations – presumably because excessive calcium was toxic to PMD cells – suggesting that fos activation is probably NOT mediated via a stretch-activated calcium channel. Medium conditioned by the mechanically injured monolayers was not able to induce fos expression, indicating that a soluble factor is likely not responsible for PMD-induced fos expression; the point being that presumably fos is activated by PMD itself. In addition, Grembowicz et al. strongly propose that PMD induces translocation of the transcriptional activator NF-κB into the nucleus of mechanically injured endothelial and smooth muscle cells.

Hubmayr RD, Shore SA, Fredberg JJ, Planus E, Panettieri RA Jr, Moller W, Heyder J, Wang N (1996) Pharmacological activation changes stiffness of cultured human airway smooth muscle cells. Am J Physiol 271 (40): C1660–1668

Human airway smooth muscle cells (HASM) were submitted to magnetic twisting cytometry (MTC). In this technique ferromagnetic beads are coated with a ligand and bound to the cell surface through integrins – a family of transmembrane heterodimers that form part of the cellular cytoskeleton. The beads are then magnetized. Subsequent application of an external magnetic field applies a torque (or twisting stress). Bead rotation is opposed by reaction forces generated within the cytoskeleton to which the bead is bound. MTC measures the applied twisting stress and the resulting angular rotation of the magnetic bead and expresses the ratio as a cell stiffness. This stiffness is a measure of the ability of the cell to resist distortion of shape. Using this technique Wang et al. [15] demonstrated that (1) endothelial cells stiffen with increasing magnitude of imposed twisting stress and that this twisting stress is transmitted across the cell membrane to the cytoskeleton via the agency of integrins and (2) the resistance of the bead to rotation is conferred by the recruitment of adhesion proteins such as talin, vinculin and actin – cytoskeletal network proteins.

In this most recent paper, the group demonstrated that HASM cell stiffness increased when cells were exposed to contractile agonists, previously documented to have an effect on actin-myosin interactions. Contractile agonists utilized in this study mediated their action by increasing intracellular calcium concentration and thereby activating the contractile apparatus. Changes in cells stiffness were also found to be dependent on the density of the matrix upon which they were plated. This suggested an important role for, not only actin-myosin interactions, but also changes in the tension on the stress fibres that link cells to adhesion plaques and changes in cellular adhesion properties related to the degree of cell spreading. The significance of these findings relates to the suggestion that shape-dependent differences in actin-myosin overlap may be mediated via shape-specific induced differences in receptors or signal transduction protein densities.

Pugin J, Dunn I, Jolliet P, Tassaux D, Magnenat JL, Nicod LP, Chevrolet JC (1998) Activation of human macrophages by mechanical ventilation in vitro. Am J Physiol 275: L1040-L1050

A plastic "lung" made of transparent plexiglas was designed to reproduce the cyclic pressure-stretching regimen induced by mechanical ventilation. The bottom chamber was made of a Bioflex Silastic, distensible and optically clear membrane. The plastic lung was connected at one end to a pressure transducer and at the other end to a regular intensive care adult ventilator.

The authors reported that alveolar macrophages (AM) were responsible for the highest degree of response to ventilator-induced lung injury. The authors did not document any comparisons with other cell types but state this was gauged by measuring secretion of interleukin 8 (IL-8) – a powerful chemotractant for neutrophils. Tumour necrosis factor-α (TNF-α) and IL-6 have also been noted to be present in BAL specimens from patients with ARDS and are thought to play a key role in ventilator-induced lung injury. In this study, they were produced in response to lipopolysaccharides (LPS) but not by mechanical ventilation.

In this study, the authors show that, in addition to pro-inflammatory mediators, a pressure stretch stimulus induced de novo synthesis of matrix metalloprotein-9 (MMP) in AMs. MMPs are enzymes that degrade extracellular matrix components and basement membranes; they are found in elevated levels in patients with ARDS. These enzymes are thought to be important to the process of lung remodelling. Specifically, MMP is a type IV collagenase.

Monocyte-derived macrophages (MDMs) produced similar responses to AM and were utilized as surrogate cells for AMs. The transcription regulator NF-κB was shown to undergo activation and nuclear translocation in MDMs submitted to cyclic pressure stretching. NF-κB contains a DNA "shear-stress" response element and it has been implicated as a critical factor for the activation of various factors including IL-8 and MMP. TNF-α and IL-6 both have NK-κB consensus DNA sequences in their promoter regions.

Ranieri MV, Suter PM, Tortorella C, De Tullio R, Dayer JM, Brienza A, Bruno F, Slutsky AS (1999) Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: a randomized controlled trial. JAMA 282 (1): 54–61

This study was a randomized controlled trial of 44 patients from the intensive care units of two European hospitals. Patients were randomized to either a control group of conventional ventilatory strategy ($n = 19$): tidal volume (Vt) to obtain normal values of arterial carbon dioxide tension (35–40 mmHg) and positive end-expiratory pressure (PEEP) to produce the greatest improvement in arterial oxygenation saturation without worsening hemodynamics; or a lung protective strategy group ($n = 18$): Vt and PEEP based on the volume-pressure curve. BAL and blood samples were obtained at the beginning of the study and were repeated at 24–30 h and 36–40 h.

Ranieri et al. found that concentrations of pro-inflammatory cytokines were similar in both groups at randomization. However, patients in the control group had an increase in BAL and plasma concentrations of IL-1, IL-6 and IL-1 receptor agonist. TNF-α, IL-6 and TNF-α receptor levels were also increased in the control group. Patients in the lung protective strategy group had reductions in both their BAL concentrations of polymorphonuclear cells, TNF-α, IL-1β, soluble TNF-α receptor 55 and IL-8; and in plasma and BAL concentrations of IL-6, soluble TNF-α receptor 75 and IL-1 receptor antagonist.

Discussion

The observation that mechanical ventilation per se can alter lung cytokines and gene expression has several implications for the practising intensivist. First, this may explain the importance of ventilatory protective strategies in improving outcomes in patients with ARDS. This can be understood by recognizing that cells have the ability to respond to a mechanical stimulus by de novo gene expression and generation of second messenger pathways – designed to protect cells from injury. The generation of this compensatory response is at the core of the local pulmonary inflammatory changes which account for biotraumatic aspects of ventilator-induced lung injury. Second, despite the development of innovative protective ventilatory strategies that minimize the fraction of inspired oxygen and limit alveolar overdistension and shear stress, many patients with ARDS go on to die from MSOF.

This observation supports the concept that ventilator-induced lung injury is an active participant in the series of cascading inflammatory changes. As compartmentalization of the local pulmonary response is lost, systemic release of inflammatory mediators promotes the massive inflammatory response that underlies MSOF [3, 4, 16, 17, 18]. This is rapidly followed by the generation of an equally dramatic compensatory anti-inflammatory reaction that is designed to down-regulate and attenuate the pro-inflammatory response. Loss of appropriate immune modulation, or persistence of inflammatory injury, appears to be involved in the inability of organisms to bring about resolution of the pro-inflammatory response and ultimately death [8, 9]. If correct, this conceptualization of ventilator-induced lung injury could lead to a paradigm shift in which therapies

to prevent ventilator-induced lung injury are not solely based on limiting mechanical injury but also limiting and/or modulating the immune response. It is incumbent upon the intensivist to understand the underlying cellular mechanisms characterizing cellular response to injury so that a rational utilization of new treatment strategies may be applied in the treatment of the critically ill.

The primary goal of immune-modulating strategies is to abrogate the inflammatory response. The consequence of over-suppression, however, is a scenario of overwhelming infection. Increased knowledge of the role of soluble and cell-based mediators has led to the development of biological response modification strategies that – by virtue of their specificity – are expected to obviate the problem of non-specific immune-response suppression. Herein lies the significance of the papers reviewed in this article. Each individual study highlights important steps in the genesis of the immune/injury response and consequently identifies key areas for potential therapeutic interventions directed towards either arresting or reversing the process of tissue injury and damage.

Much of the effort expended on immuno-therapy – in the setting of sepsis and MSOF – has focused on anti-cytokine strategies. Interest in cytokine inhibition or blockade was stimulated by the finding that polyclonal antibodies to TNF-α prevented death induced by endotoxaemia in mice [9]. Initial laboratory and in vitro models suggested that anti-TNF-α strategies provided complete protection against shock and vital organ dysfunction when administered prior to the onset of a lethal insult and significantly attenuated sepsis-induced lung injury by aborting the disruption of the vascular endothelium [9]. Unfortunately, results from animal and human studies have not been as successful. The NORASEPT II study group recently published a double-blind randomized controlled trial of monoclonal antibody to human TNF-α in the treatment of 1879 adult patients with septic shock. They were unable to demonstrate a clinically significant difference in 28-day mortality between placebo and anti-TNF-α [19]. Other studies of inhibition of cytokines (i.e., IL-1) and chemokines (i.e., IL-8) have demonstrated positive results in animal models [9], but translation to humans has been problematic. Notwithstanding the disappointing results of anti-cytokine therapy in patients with sepsis and MSOF, it must be noted that no study, to our knowledge, has looked specifically at the clinical efficacy of anti-cytokine therapy in preventing MSOF in patients with ARDS.

In addition to anti-cytokine therapies, the development of techniques for manipulating nucleic acids and strategies for delivering DNA to humans has made gene therapy a reality. Critical illness is probably a good target for gene therapy because of the high mortality and need for only transient treatments. Genes can be delivered in vivo using viral vectors (replication-deficient adenoviruses and adenovirus-associated viruses) or non-viral vectors (liposomes, direct DNA injection and polycation DNA glycoconjugates). The practical advantage of gene therapy is its ability to target therapies to individual tissues or cell types, to produce proteins locally that can act intra-cellularly or in an autocrine, juxtacrine or paracrine fashion, and sustain new protein production for periods of up to several weeks with a single administration. Oligonucleotide therapy also offers an interesting therapeutic alternative. The approach in this strategy is to halt DNA transcription or mRNA translation with code blocking, triple-helix forming or "anti-sense" oligomers. To illustrate the potential applications of gene therapy in the management of ALI, Brigham and Stecenko used a vector system that over-expressed the prostaglandin synthase gene in an in-vivo model of ALI. This resulted in increased production of prostaglandin E2 and prostacyclin in the in-vivo lung. The authors postulate that increased expression of prostaglandin E2 and prostacyclin protected the lung from the effects of endotoxin [20].

Furthermore, increased understanding of tissue-specific promoter regions and of mechanisms controlling the regulation of gene expression offer potential for close control of therapeutic gene expression within the lung. Other promising therapeutic genes would include genes encoding for anti-oxidant enzymes, anti-proteases or genes activated by mechanical stresses responsible for the generation of the immune response seen in ventilator-induced lung injury. Hence, knowledge from the three basic science papers presented above provide clues to interesting future therapies. Anti-sense fos, over-expression of inhibitor of NKκB and modulators of actin-myosin interaction may play an important role in the future, alone or as combination therapy, in the prevention of some biological consequences of mechanically induced lung injury and consequently in the generation and propagation of the immune response underlying MSOF in ventilated patients with ARDS.

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