Critical Care Perspective

On the Physiologic and Clinical Relevance of Lung-born Cytokines during Ventilator-induced Lung Injury

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Management of acute respiratory distress syndrome (ARDS) has improved in recent years, resulting in a decrease in mortality (1, 2), although precise reasons for this are still debated. Physiologic and clinical research has been directed at determining whether the lesions produced by injurious ventilation in laboratory animals, termed “ventilator-induced lung injury” (VILI) (3), have their counterpart in humans called ventilator-associated lung injury (4). This work recently culminated in the demonstration of a 22% reduction in mortality of patients with ARDS ventilated with a reduced Vt. The reason why such a simple strategy reduces mortality is not obvious but, in any case, it is not related to gross barotrauma reduction (5). Investigators suggest that it might be due to less lung and systemic inflammation, as attested by lower plasma interleukin (IL)-6 concentrations in patients ventilated with a low Vt (5). This is in keeping with clinical findings of lower bronchoalveolar lavage (BAL) fluid and serum cytokine concentrations in patients ventilated with 7.6 ml/kg compared with 11.1 ml/kg Vt (6). Moreover, the possibility that multiple-system organ failure frequently observed in patients with ARDS could be caused by inappropriate ventilator settings has been explicitly raised (7–9).

It is also quite possible that changes in cytokine concentrations are unrelated to mechanical ventilation, which merely permits patients to live long enough to develop multipletsystem organ failure. Prolonged survival may provide time for complications, such as critical illness neuropathy, to develop (10, 11), whereas these disorders went undiagnosed when patients died earlier. The work that has led to the hypothesis that mechanical ventilation causes inflammation and cytokine production in intact lungs, enhances this production in damaged lungs and leads ultimately to multiple-system organ failure, should therefore be reevaluated.

Mead and colleagues (12) speculated that ARDS could be, in part, an iatrogenic disease due to the mechanical stress applied to nonuniformly expanded lungs. The subsequent demonstration that high lung stretch rapidly results in cellular lesions and permeability type pulmonary edema made this “mechanical explanation” plausible. Indeed, this condition can be produced in rats well before there is any noticeable inflammation (3).

Stretching cells also elicits a variety of responses, including entry of calcium into cells (with ensuing changes in endothelial permeability [13]), altered membrane lipid trafficking (14), and increased activity of stress-responsive genes (15) that may participate in the genesis of VILI. Severe alterations (cell lysis, basement membrane denudation [3]) may favor lung neutrophil adhesion and the activation and release of inflammatory mediators. The lungs are clearly greatly inflamed at later stages of VILI, as evidenced by their infiltration by leukocytes (16, 17). The aim of this paper is to highlight several inconsistencies in the experimental and clinical evidence of cytokine production during injurious ventilation that question the role of cytokines during VILI.

EFFECTS OF STRETCHING CELLS ON THE RELEASE OF CYTOKINES AND CHEMOKINES

Human alveolar macrophages subjected to prolonged (24 hours) cyclic stretching (considered as an in vitro analog of high volume mechanical ventilation) release IL-8, a chemokine involved in polymorphonuclear recruitment, but not cytokines such as tumor necrosis factor (TNF)-α or IL-6 (18). Concordant findings were reported with the human alveolar epithelial cell line (A459) (19). The release of the chemotactant IL-8 by isolated cells may help explain the neutrophil infiltration that occurs later during VILI (16, 17, 20–22).

These studies suggest that stretching cells that are normally present in alveoli results in the release of a chemokine involved in neutrophil recruitment. However, they did not support the acute release of the plethora of cytokines observed in some (23, 24) but not all (25) initially uninjured isolated lung preparations. Nevertheless, the fact that alveolar cells do not produce TNF-α or IL-6 gives no indication as to whether or not they are released during injurious mechanical ventilation of the whole organ.

RESPONSE OF ISOLATED LUNG PREPARATIONS TO INJURIOUS VENTILATION

Inconsistent Observations on the Local Release of Cytokines

Some experimental studies have concluded that injurious ventilation (i.e., lung overdistension and repeated “closure-reopening”) results in the production of many cytokines. For example, Tremblay and colleagues ventilated isolated, unperfused rat lungs for 2 hours with various ventilation strategies and found that all injurious strategies increased BAL TNF-α (23). This increase was greater after the most injurious ventilation (high Vt–zero end-expiratory pressure [ZEEP]). Other mediators were released, including the inflammatory cytokines IL-1β, IFN-γ, and IL-6 (which was high only when Vt was very high), the chemokine macrophage inflammatory protein (MIP)-2 (the rodent equivalent for IL-8), and also the antinflammatory cytokine IL-10. In this study (23), some animals received endotoxin before lung removal, to mimic a septic state. This pretreatment significantly increased BAL TNF-α in lungs ventilated with mildly

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Inconsistencies in Studies of Animals with Injured Lungs

However, it is difficult to understand how a noninjurious ventilation strategy (24, 30) can be so “proinflammatory”; Conflicting results have been reported on the release of inflammatory cytokines in initially intact rats (25, 29, 40). Rats ventilated with a high VT and ZEEP consistently develop severe pulmonary edema with diffuse alveolar damage (3, 25, 36, 37, 40, 41). Despite an injurious ventilation modality that increased BAL protein concentration, Verbrugge and colleagues found no local or systemic release of TNF-α (40). These results were confirmed by the same team in a different study (29). In another study from a different team, no TNF-α was detectable in BAL after 2 hours of 42 ml/kg VT ventilation, whereas IL-1β remained low (less than 50 pg/ml), but was slightly higher than in rats ventilated with 7 ml/kg VT (25). BAL MIP-2 concentrations in rats ventilated with 42 or 7 ml/kg VT were similar. In addition, TNF-α, IL-1β, and MIP-2 in plasma remained undetectable in both control animals and rats with severe VILI (25).

Inconsistent Observations on the Systemic Release of Cytokines

It has been suggested that injurious ventilation might promote cytokine decompartmentalization (i.e., translocation to the systemic circulation) in addition to lung release. This concept associates multiple-system organ failure with injurious mechanical ventilation (8, 28, 29) and is supported by some data obtained in isolated-perfused mouse lungs (24). In this study (24), high VT ventilation resulted in the release of considerable amounts of TNF-α (more than 200 pg/ml) and IL-6 (1,000 pg/ml) into the perfusate after 150 minutes. However, IL-6, although markedly lower, was also quite high (400 pg/ml) in the perfusate of preparations ventilated with a normal VT (see Figure 5B of Reference 24). These authors also showed that this production of inflammatory cytokines was mediated by the activation of nuclear factor-κB (30). Intriguingly, this study (30) found several hundred picograms per milliliter of various chemokines and cytokines (murine IL-8, macrophage chemotactic protein-1, IL-6) in the perfusate, even during normal VT ventilation (see Figures 3–5 of Reference 30). It may be tempting to conclude, as the authors did, that these two studies indicate that lung overdistension during mechanical ventilation is responsible for the release of inflammatory cytokines in the lungs, with subsequent systemic translocation. However, it is difficult to understand how a noninjurious ventilation strategy (24, 30) can be so “proinflammatory”; an observation also made in vivo (31) (see Inconsistent Observations on Systemic Release of Cytokines during Injurious Ventilation). It is also worth noting that these observations were made in a nonphysiologic environment (i.e., severe hypocapnia) because no carbon dioxide was added to the gas used for ventilation or to the perfusate of the preparation. It is thus difficult to believe that these findings (activation of nuclear factor-κB and cytokine release) were entirely due to overdistension. The fact that ventilation with a normal VT also led to cytokine release suggests that hypocapnia itself has a proinflammatory effect. The recent demonstration of the deleterious effect of hypocapnic alkalosis during ischemia–reperfusion injury in isolated lungs is in keeping with this interpretation (32). Although this effect has no clear explanation, the authors (32) speculated that there was a continuum between the protective effect of hypercapnia (33) and the worsening effect of hypocapnia. Hypercapnia also seems to protect lungs against microvascular permeability alterations due to overinflation (34, 35).

Inconsistent Findings on the Capacity of Some Modes of Ventilation to Promote Cytokine Release In Vivo

This is a most controversial issue. A key point is that it is often not possible to reproduce typical ex vivo experiments in intact animals. For instance, mechanical ventilation with a very high VT for a period of less than 1 hour results in extremely severe lung injury that is rapidly lethal in rats (36, 37). Total lung collapse and reexpansion, as produced in isolated lungs ventilated with ZEEP, is also unrealistic. Thus, the conditions that have led to some of the observations made in ex vivo preparations may never be encountered in vivo. In addition, cardiopulmonary interactions are not taken into account under ex vivo conditions and may complicate interpretation, raising such questions as to whether the systemic increase in a mediator in animals with severe cardiopulmonary failure reflects lung injury or shock.

With these limitations in mind, it is clear that any hypothesis proposed by studies on isolated organs should be verified in vivo before drawing any inference on their physiologic relevance. Interestingly, as detailed below, there are consistent results on the absence of TNF-α release during injurious ventilation of intact animals and inconsistent results concerning the same TNF-α release in preinjured animals. Similarly, some authors reported increased TNF-α messenger RNA in intraalveolar cells during injurious ventilation (38), whereas others did not confirm these findings, despite severe VILI (39).

Negligible Effects of Injurious Ventilation on TNF-α and Other Proinflammatory Mediators Released in Intact Animals

Two independent teams showed that highly injurious ventilation does not affect acute in vivo lung inflammatory cytokine production in initially intact rats (25, 29, 40). Rats ventilated with a high VT and ZEEP consistently develop severe pulmonary edema with diffuse alveolar damage (3, 25, 36, 37, 40, 41). Despite an injurious ventilation modality that increased BAL protein concentration, Verbrugge and colleagues found no local or systemic release of TNF-α (40). These results were confirmed by the same team in a different study (29). In another study from a different team, no TNF-α was detectable in BAL after 2 hours of 42 ml/kg VT ventilation, whereas IL-1β remained low (less than 50 pg/ml), but was slightly higher than in rats ventilated with 7 ml/kg VT (25). BAL MIP-2 concentrations in rats ventilated with 42 or 7 ml/kg VT were similar. In addition, TNF-α, IL-1β, and MIP-2 in plasma remained undetectable in both control animals and rats with severe VILI (25).

Inconsistencies in Studies of Animals with Injured Lungs

Conflicting results have been reported on the release of inflammatory mediators by preinjured lungs subjected to injurious ventilation. Imai and colleagues (42) studied the effect of mildly injurious ventilation (VT of 12–15 ml/kg) on BAL TNF-α in surfactant-depleted rabbits. TNF-α markedly increased during the 4 hours of mechanical ventilation, rising from 100 to 6,700 pg/ml. In contrast, using the same lung lavage model in rats instead of rabbits but with an even more deleterious ventilation protocol, Verbrugge and colleagues found no TNF-α in BAL (40). Similarly, BAL IL-1β concentrations were comparable in surfactant-depleted rabbits ventilated with low or high VT (43). Chiumello and colleagues (44) found that TNF-α and MIP-2 concentrations were similar in the lung edema fluid recovered by aspiration in rats with hydrochloric acid lung injury, whether the strategy was mildly injurious (VT 16 ml/kg, ZEEP) or protective (VT 9 ml/kg, positive end-expiratory pressure [PEEP] 5 cm H2O).
Conflicting results have also been reported with intratracheal administration of anticytokines in surfactant-depleted rabbits ventilated with a high Vt. Administration of an IL-1 receptor antagonist reduced lung albumin, elastase, and neutrophil count but failed to reduce lung lesions and to prevent decline in compliance and oxygenation (43), whereas an anti–TNF-α antibody nearly abolished lung lesions (42). It is difficult to understand how a single intervention may nearly abrogate the complex manifestations of VILI.

Inconsistent Observations on Systemic Release of Cytokines during Injurious Ventilation

In the above-mentioned study by Chiumello and colleagues (44), plasma TNF-α and MIP-2 concentrations were higher after high-Vt–ZEEP ventilation. The authors concluded that a particular ventilation strategy could affect the release of cytokines into the circulation, leading eventually to multiple-system organ failure. Surprisingly, and left unexplained, all high-Vt–ZEEP animals survived the entire experiment, whereas animals of a third group ventilated with a low Vt (9 ml/kg) and ZEEP rapidly developed fatal shock. Paradoxically, these animals had the lowest serum cytokine levels of all (see Figure 7 of Reference 44).

Even more unanticipated was the recent report that a protective lung strategy (31) might cause, in vivo, the release of TNF-α into the circulation, an observation made in isolated mouse lung control animals, leading to increased mortality in this group (57). It is not very surprising that the only prospect of reducing cytokines is doubtful unless a release of TNF-α may, in fact, be due to a relatively high Vt (12 ml/kg) used in control animals, leading to increased mortality in this group (57). This was further suggested by a metaanalysis of available clinical trials (58). This metaanalysis also suggested that the use of a very low Vt (6 ml/kg or less) may be associated with increased mortality as compared with a moderately reduced Vt (58) (despite the fact that one experimental study suggested that very low Vt may have a beneficial effect on pulmonary edema [59]).

Given the uncertainties about the benefits of drastic Vt reduction on mortality, and the hesitation as to whether large Vt favors inflammation or antiinflammation (see below), to reduce Vt in the only prospect of reducing cytokines is doubtful unless a precise response curve linking Vt reduction on the one hand to cytokine release and survival on the other is established (which seems neither feasible nor desirable).

Finally, a recent clinical study evaluated BAL and plasma cytokine changes according to ventilation strategy (60). Cytokines were significantly increased in BAL fluid and in plasma when patients were switched from a low Vt (5 ml/kg)–high PEEP (15 cm H2O) to a high Vt (12 ml/kg)–low PEEP (5 cm H2O) strategy. An accompanying editorial (61) underlined the fact that the mediators released were principally antiinflammatory (IL-1 RA and IL-10), whereas TNF-α was only moderately elevated and IL-1 β remained undetectable. It is also difficult to understand why changes in concentration occurred so rapidly (plasma cytokine concentrations increased less than 1 hour after ventilatory strategy shift and their half-life was surprisingly short) (61).

In conclusion, as clinical studies reflect some of the inconsistencies observed in experimental ones, it is impossible to know whether ventilation affects the balance of cytokines toward inflammation or antiinflammation (26, 61). In addition, the response of lungs “primed” by the multiple events that occur in critically ill patients (infection, shock, transfusion, etc.) is probably very complex. Basing any pathophysiologic concept and a fortiorti therapeutic intervention (8, 54) on these results is premature and potentially hazardous.

EFFECTS OF DIFFERENT VENTILATION MODALITIES ON THE REGIONAL AND SYSTEMIC RELEASE OF INFLAMMATORY MEDIATORS IN PATIENTS WITH ARDS

In the ARDSnet trial, mortality was considerably lower in patients ventilated with 6 ml/kg rather than 12 ml/kg predicted body weight Vt (5). Plasma IL-6, IL-8, and IL-10 decreased in both groups of patients but to a further extent in those treated with low Vt (52). Although this greater reduction was significant, it remained modest (IL-6 concentration was 350 pg/ml on Day 1 and 110 pg/ml on Day 3; these values were 110 and 85 pg/ml for IL-8 and 35 and 26 pg/ml for IL-10, on Days 1 and 3, respectively). Ranieri and colleagues reported significant decreases in BAL and plasma concentrations of many inflammatory and antiinflammatory mediators (TNF-α, IL-1β, IL-6, IL-8, soluble TNF receptors, IL-1 RA) in patients ventilated with a lung protective strategy consisting in a 7.6 ml/kg Vt with 14.8 cm H2O PEEP) as compared with those ventilated with 11.1 ml/kg Vt and 6.5 cm H2O PEEP). This study was not intended to draw any correlation between patient outcome and cytokine profile (53, 54). BAL polymorphonuclear leukocytes of the 11.1 ml/kg Vt group were later found to be activated (55).

It was recently argued that the apparent improvement in mortality with very low Vt ventilation (5, 56) (6 ml/kg or less) may, in fact, be due to a relatively high Vt (12 ml/kg) used in control animals, leading to increased mortality in this group (57). This was further suggested by a metaanalysis of available clinical trials (58). This metaanalysis also suggested that the use of a very low Vt (6 ml/kg or less) may be associated with increased mortality as compared with a moderately reduced Vt (58) (despite the fact that one experimental study suggested that very low Vt may have a beneficial effect on pulmonary edema [59]). Given the uncertainties about the benefits of drastic Vt reduction on mortality, and the hesitation as to whether large Vt favors inflammation or antiinflammation (see below), to reduce Vt in the only prospect of reducing cytokines is doubtful unless a precise response curve linking Vt reduction on the one hand to cytokine release and survival on the other is established (which seems neither feasible nor desirable).

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IMPACT OF CYTOKINE RESEARCH ON THE CLINICAL MANAGEMENT OF PATIENTS WITH ARDS

Clinicians must remain cautious when trying to apply the elusive concept of ventilation and inflammation interaction to patient care for the following reasons. First, improvement in ARDS
prognosis has had little (if anything) to do with our increasing knowledge of cytokines. Indeed, Vt reduction was based on classic cardiopulmonary physiology premises (1, 2, 12, 57) and probably helped improve prognosis well before any randomized controlled trial was performed (5, 58). Second, multiple organ failure could result more from uncontrolled infection, as postulated initially (62), than from persistent inappropriate ventilator settings. For example, ventilator-associated pneumonia occurs in more than 50% of patients with ARDS (63–65) and is associated with a very high mortality rate especially when diagnosis is delayed and/or antibiotic treatment inadequate (66). Third, the finding of small changes in lung or plasma cytokine concentrations (5–7) during protective lung ventilation is in no way indicative of a causal link with outcome (see above).

Moreover, lung injury, multiple-system organ failure, and shock are interconnected. Mechanical ventilation (especially when Vt–high airway pressure strategies are used) impairs hematodynamics and may favor organ dysfunction. On the other hand, the contributory role of cytokines is perhaps overstated and more work is needed to determine to what extent they function as an amplifying factor. The numerous contradictions and inconsistencies detailed in this article are particularly worrisome to someone wishing to have a clear view of the role of lung-borne cytokines during ventilator-associated lung injury (4). The physiologic and clinical research effort that culminated in the demonstration of the beneficial role of Vt reduction (67) during ARDS must receive a well merited acknowledgment. Indeed, it occurred in a context of strong incentive to reorientate research toward an apparently more fundamental approach (68).

The recent suggestion of genetic predisposition to VILI further complicates the issue (69, 70). The difficulty of such an approach, the remoteness of its potential use, and its probable cost should be compared with the simplicity and usefulness of reducing lung stretch during mechanical ventilation, as elaborated by Mead (12). This strongly favors individual bedside tailoring of mechanical ventilation according to lung mechanics (71–74). The contention of this Critical Care Perspective is not that research on cytokines, genomics, or gene therapy is not worth doing. Obviously, considerable medical progress will ensue. However, temporerative enthusiasm led to numerous medical catastrophes, both in critical care (75) and in other fields of medicine (consider, for instance, the problems associated with some gene therapy trials [76]). Norman C. Staub recently wrote “A serious malaise has overtaken physiology. Physiology has become so bedazzled by molecular biology that physiologists are not producing new physiological insights . . . . Despite fundamental discoveries, there is an enormous gap separating molecular biology from the treatment of disease. Few of the touted applications work. Some have unexpected, even dangerous consequences.” (77). The negative results of anticytokine clinical trials in sepsis (78, 79) should make physicians wishing to use them to prevent ventilator-associated lung injury (8, 23) very cautious. Otherwise, we may return to a form of medicine prac-

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