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Mechanical ventilation modulates Toll-like receptor signaling pathway in a sepsis-induced lung injury model

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Abstract *Background:* Experimental and clinical studies on sepsis have demonstrated activation of the innate immune response following the initial host–bacterial interaction. In addition, mechanical ventilation (MV) can induce a pulmonary inflammatory response. How these two responses interact when present simultaneously remains to be

elucidated. We hypothesized that MV modulates innate host response during sepsis by influencing Toll-like receptor (TLR) signaling.

Design: Prospective, randomized, controlled animal study. *Subjects:* Male, septic Sprague–Dawley rats. *Interventions:* Sepsis was induced by cecal ligation and perforation. At 18 h, surviving animals had the cecum removed and were randomized to spontaneous breathing or two strategies of MV for 4 h: high (20 ml/kg) tidal volume (V_T) with no positive end-expiratory pressure (PEEP) versus low V_T (6 ml/kg) plus 10 cmH₂O PEEP.

Measurements and main results: Histological evaluation, TLR-2, TLR-4, inhibitory kappaB alpha ($I\kappa B\alpha$), interleukin-1 receptor-associated kinase-3 (*IRAK-3*) gene expression, protein levels and immunohistochemical lung localization, inflammatory cytokines gene expression, and protein serum concentrations were analyzed. MV with low V_T plus PEEP attenuated sepsis-associated TLR-4 activation, and produced a significant decrease of *IRAK-3* gene expression and protein levels, a significant increase of $I\kappa B\alpha$, and a decrease in lung gene expression and serum levels of cytokines. High- V_T MV caused a significant increase of TLR-4 and *IRAK-3* protein levels, lung and systemic cytokines,

and mortality, and a significant decrease of $I\kappa B\alpha$.

Conclusions: Our findings suggest a novel mechanism that could partially explain how MV modulates the innate immune response in the

lung by interfering with cellular signaling pathways that are activated in response to pathogens.

Keywords Acute lung injury · Sepsis · Positive-pressure ventilation · Cytokine · Toll-like receptor

Introduction

Mechanical ventilation (MV) is essential for the management of many patients with sepsis-induced lung injury; however, evidence from experimental and clinical studies has demonstrated that certain MV strategies can cause or worsen lung injury [1–6]. Although the Acute Respiratory Distress Syndrome (ARDS) Network trial demonstrated that lowering tidal volumes (V_T) delivered to patients with acute lung injury (ALI) reduced mortality [7], the molecular mechanisms underlying this outcome remain to be elucidated.

Lung cells express a large repertoire of genes under transcriptional control of biomechanical forces [8, 9] and bacterial infections [10]. Lipopolysaccharide (LPS) has a major role in the devastating nature of sepsis, in part by binding to cell-surface receptors exerting pro-inflammatory effects. Toll-like receptors (TLRs) detect host invasion by pathogens and constitute the key link between the innate and adaptive immune responses [11, 12]. TLR signaling is regulated through a series of intracellular proteins including the interleukin (IL)-1 receptor-associated kinases (IRAK). Downstream in the TLR-4 activation, the I κ B kinase (IKK) complex catalyzes phosphorylation of inhibitory κ B ($I\kappa$ B) proteins [13]. This causes degradation of cytoplasmic inhibitors of nuclear factor- κ B (NF- κ B) and nuclear translocation of NF- κ B. NF- κ B is normally sequestered in the cytoplasm through association with $I\kappa$ B α . After cellular stimulation, $I\kappa$ B α is degraded, allowing NF- κ B to move to the nucleus, where it binds to specific promoter sequences and initiates transcription, resulting in expression of cytokines such as IL-6 and tumor necrosis factor- α (TNF- α) and other gene products [14–18]. There are four distinct human genes encoding for the four IRAK proteins. IRAK-3 (also known as IRAK-M) inhibits NF- κ B activation and negatively regulates TLR signaling [19]; it is a mediator of LPS-induced tolerance [20] and a selective inhibitor of the classical [19] and alternative NF- κ B pathway [21].

Held et al. [22] reported that inflammatory responses induced by high- V_T MV were similar to those evoked by LPS, and Moriyama et al. [23] postulated that high- V_T MV enhances the LPS recognition pathway via upregulation of CD14. Therefore, we hypothesized that MV can modulate the TLR/IRAK-3 signaling pathway in lung during sepsis. We evaluated this hypothesis by comparing

protective versus injurious MV in an animal model of sepsis-induced ALI.

Methods

Animal preparation

The protocol was approved by Hospital Universitario N.S. de Candelaria Research Committee and the Committee for the Use and Care of Animals, University of La Laguna (Tenerife, Spain). We studied 40 male Sprague–Dawley rats weighing 300–350 g. Animals were anesthetized by intraperitoneal injection of 50 mg/kg body weight ketamine hydrochloride and 2 mg/kg body weight xylazine. Sepsis was then induced by cecal ligation and perforation (CLP) [24]. A detailed description of this experimental model is provided elsewhere [4]. Eighteen hours after CLP, the peritoneal cavity was reopened in surviving animals, and the cecum was then excised distal to the ligature and removed. After closing the abdomen, each animal received 10 ml normal saline subcutaneously for fluid resuscitation. Then, we performed a tracheotomy using a 14-G Teflon catheter. Thereafter, animals were paralyzed with 1 mg/kg pancuronium bromide and connected to a time-cycled, volume-limited rodent ventilator (Ugo Basile, Varese, Italy).

Experimental protocol

Following surgical procedures, surviving septic rats were randomly divided into three groups: (1) control: anesthetized, spontaneously breathing, (2) low- V_T group: ventilated with 6 ml/kg plus 10 cmH₂O positive end-expiratory pressure (PEEP), and (3) high- V_T group: ventilated with 20 ml/kg and zero PEEP. FiO₂ was 60%. In pilot studies we monitored animals invasively and found comparable blood gases at the end of the 4-h ventilation period using FiO₂ = 0.60 (PaO₂ 141 ± 21 versus 159 ± 29 mmHg and PaCO₂ 43 ± 4 versus 39 ± 3 mmHg, for the low- V_T plus PEEP and high- V_T groups, respectively; n = 5 rats/group). As such, during our experimental protocol, animals were monitored noninvasively. Respiratory rate was set to maintain constant minute ventilation in both groups. These settings were maintained

for 4 h, while anesthetized and paralyzed, supine on a restraining board inclined 20° to the horizontal.

Histological examination

At the end of the 4-h ventilation period, a midline thoracotomy/laparotomy was performed in the first six surviving rats from each group and the abdominal vessels were transected. The heart and lungs were removed en bloc. The lungs were isolated from the heart, the trachea was cannulated, and the right lung was fixed by intratracheal instillation of 3 ml 10% neutral buffered formalin. After fixation, the lungs were floated in 10% formalin for 1 week. Lungs were then serially sliced from apex to base and specimens were embedded in paraffin, then cut (3 μ m thickness), stained with hematoxylin–eosin, and examined under light microscopy [see Electronic Supplementary Material (ESM) for details].

RNA extraction and reverse transcription

Left lungs were excised, washed with saline, frozen in liquid nitrogen, and stored at -80°C for subsequent RNA extraction. Lungs were homogenized, and total lung tissue RNA was extracted using TRIreagent (Sigma, Hamburg, Germany) and DNase I digestion (Amersham Biosciences, Essex, UK) [25, 26]. Methods for expression levels of tumor necrosis factor- α ($TNF-\alpha$), interleukin-6 ($IL-6$), and $IRAK-3$ genes by real-time polymerase chain reaction (PCR) are described in the ESM.

Cytokine serum levels

At the end of the 4-h experimental period, 2 ml blood was collected from the six surviving rats in each group by cardiac puncture and centrifuged for 15 min at 3,000 rpm. Sera were divided into aliquot portions and frozen at -80°C . $TNF-\alpha$ and $IL-6$ serum protein concentrations

were measured by enzyme-linked immunosorbent assay (ELISA) (see ESM for details).

Western immunoblotting

Detection of TLR-2, TLR-4, $I\kappa B\alpha$, and $IRAK-3$ protein expression was performed by Western blotting, as described in the ESM.

Immunohistochemistry for $IRAK-3$

Immunohistochemical stains were performed by applying a standard avidin–biotin complex technique. Protein was visualized using 3-amino-9-ethylcarbazole as substrate (red-pink color), and nuclei were lightly counterstained using hematoxylin (see ESM for details).

Statistical analysis

Statistical analysis was performed with the Fisher exact test and paired and unpaired Student's t tests. Comparisons involving all groups were performed with one-way analysis of variance. If a difference was found, Student's t test was applied. Values derived from cytokine gene expression were expressed as group median, normalized by the lowest level in the group, and tested with the Kruskal–Wallis test and the Mann–Whitney U test. Data from ELISA were analyzed by the Student–Newman–Keuls pairwise multiple-range test. Data analysis was performed using SPSS 15.0 (SPSS Inc., Chicago, IL). A value of $p < 0.05$ was considered significant.

Results

Ten rats died from sepsis after CLP; the remaining 30 animals were randomly allocated to the three study

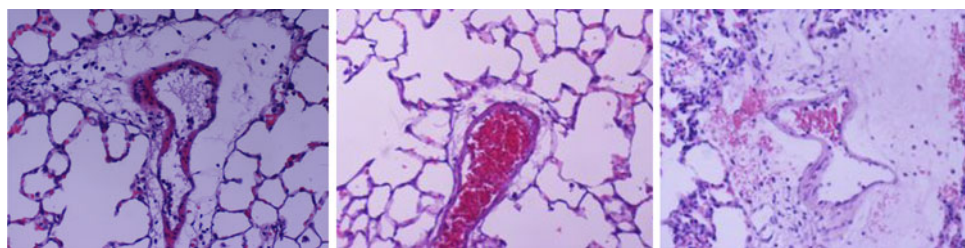


Fig. 1 Representative histopathological features of septic lungs after 4 h of spontaneous breathing or mechanical ventilation. *Left panel* lung from a septic, spontaneous breathing animal showing pulmonary infiltrates and perivascular edema. *Middle panel* lung from a septic animal after 4 h of mechanical ventilation with low tidal volume plus 10 cmH_2O PEEP showing occasional cellular

infiltrates, reduced edema, and relatively normal lung architecture. *Right panel* lung from a septic animal after 4 h of mechanical ventilation with high tidal volume showing pulmonary infiltrates, perivascular edema, and derangement of lung architecture (hematoxylin–eosin, $\times 200$)

groups. After cecum removal, four animals died in the spontaneous breathing group. No animals ventilated with low V_T and PEEP died during MV, but two out of ten animals ventilated with high V_T died within the same experimental period ($p = 0.042$). Respiratory rate was 90 ± 1 cycles/min in the low- V_T group and 30 ± 1 in the high- V_T group. Mean peak inspiratory pressure was 28 ± 1 cmH₂O in the high- V_T group. Animals ventilated with high V_T had histological evidence of lung injury (Fig. 1), with the highest histological scores (11.5 ± 2.0), whereas animals ventilated with low V_T plus PEEP had a histological score that was lower than in septic, spontaneous breathing animals (2.2 ± 0.5 versus 4.5 ± 0.3 , respectively) ($p < 0.0001$).

Cytokine gene expression and protein levels

TNF- α and *IL-6* were expressed in lung homogenates of all septic rats (Fig. 2a). High V_T increased *TNF- α* and *IL-6* gene expression compared with spontaneously breathing animals. Low V_T plus PEEP caused similar *IL-6* gene expression as spontaneously breathing animals but decreased *TNF- α* gene expression compared with unventilated animals ($p < 0.001$) (Fig. 2a). After 4 h of high V_T , *TNF- α* and *IL-6* serum concentrations were higher than in unventilated animals (166 ± 31 versus 89 ± 17 pg/ml, $p < 0.001$; $2,209 \pm 153$ versus 801 ± 86 pg/ml, $p < 0.0001$, respectively). In animals ventilated with low V_T , *TNF- α* and *IL-6* serum levels were lower than in spontaneous breathing animals (23 ± 9 versus 89 ± 17 pg/ml, $p = 0.0001$; and 426 ± 58 versus 801 ± 86 , $p < 0.0001$, respectively) (Fig. 2b, c).

TLR-4 and TLR-2 regulation and $I\kappa B\alpha$ protein levels

MV modulated TLR expression in septic lungs (Fig. 3). We did not detect changes in TLR-2 protein levels in any of the experimental groups. TLR-4 protein levels were higher in rats ventilated with high V_T than in low V_T plus PEEP or unventilated animals ($p < 0.001$). MV induced degradation of $I\kappa B\alpha$ to a greater extent in septic animals ventilated with high V_T compared with nonventilated or ventilated with low V_T plus PEEP ($p < 0.001$). MV with low V_T plus PEEP had no detectable effects on $I\kappa B\alpha$ degradation.

IRAK-3 gene expression and protein levels in the lungs

Levels of *IRAK-3* gene expression increased slightly when septic animals were ventilated with high V_T (Fig. 4a). However, MV with low V_T plus PEEP caused marked downregulation of *IRAK-3* gene expression compared with septic baseline ($p = 0.004$). *IRAK-3* gene

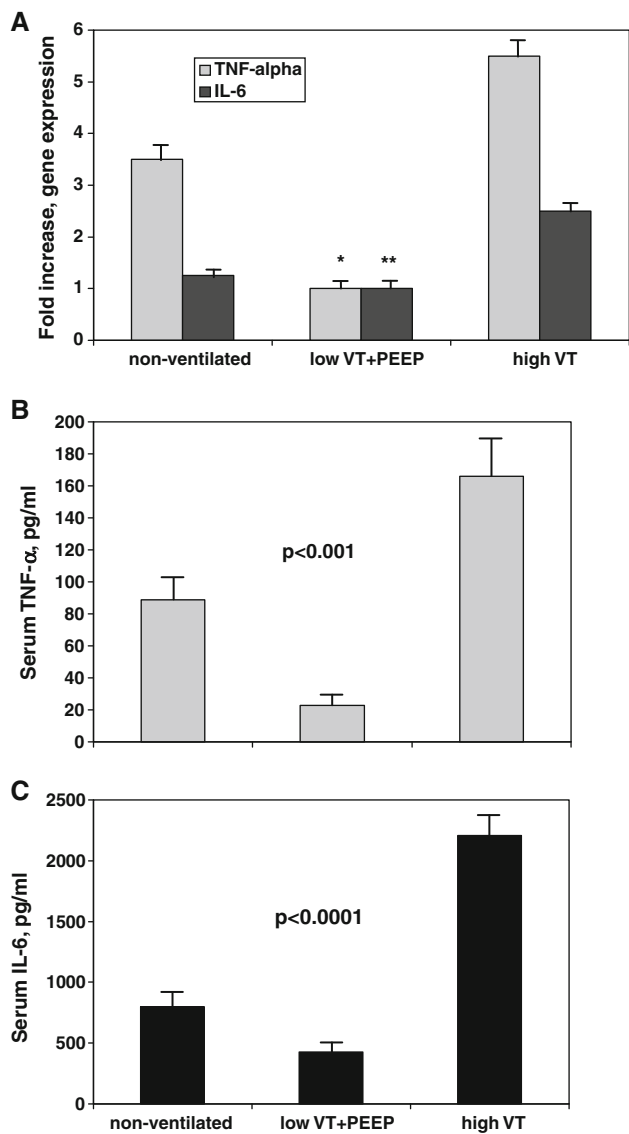


Fig. 2 a Fold changes in gene expression of *TNF- α* and *IL-6* in the lungs after 4 h of spontaneous breathing, mechanical ventilation with low V_T plus 10 cmH₂O PEEP, or with high V_T . Bars represent the median ($n = 6$ rats/group). * $p < 0.001$ (for *TNF- α* between septic versus septic/low V_T + PEEP, and septic/low V_T + PEEP versus septic/high V_T); ** $p < 0.01$ (for *IL-6* between septic/low V_T + PEEP versus septic/high V_T). b, c Effect of 4 h of spontaneous breathing in nonventilated, anesthetized animals and of 4 h of mechanical ventilation with low V_T and high V_T on systemic protein levels of *TNF- α* (a) and *IL-6* (b). Bars represent mean values ($n = 6$ rats/group)

expression during low- versus high- V_T MV was statistically different ($p = 0.003$). *IRAK-3* protein levels in the lungs were downregulated in animals ventilated with low V_T plus PEEP compared with those unventilated ($p < 0.01$). However, *IRAK-3* protein levels were higher after 4 h of high V_T as compared with all groups ($p < 0.001$) (Fig. 4b).

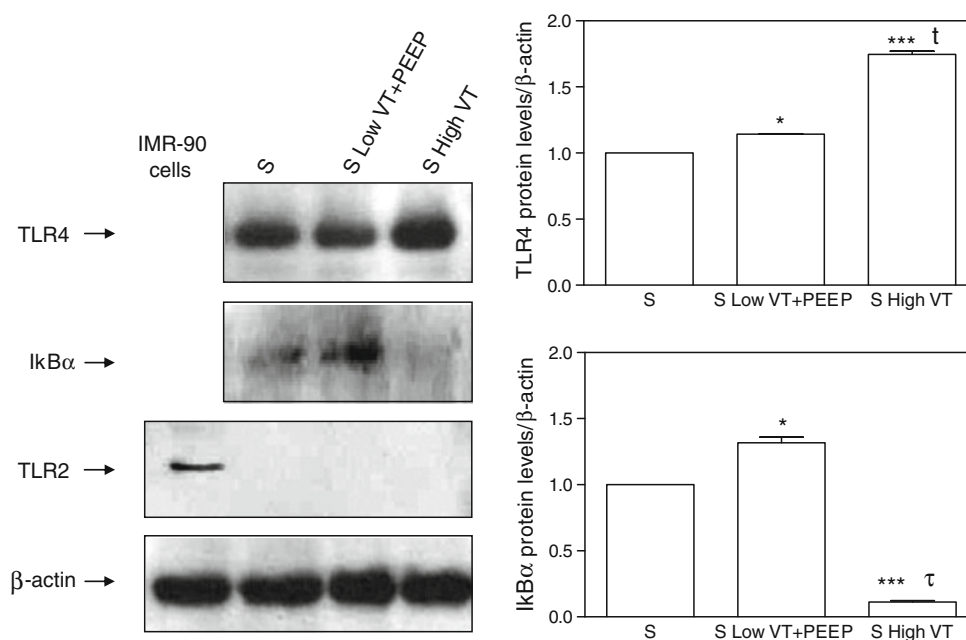


Fig. 3 Western blotting showing the effects of mechanical ventilation on TLR-4, TLR-2, and I κ B α protein levels in several groups of septic animals: spontaneous breathing, ventilated with low tidal volume plus 10 cmH₂O PEEP, and ventilated with high tidal volume for 4 h. Bars represent densitometric analysis [mean \pm standard deviation (SD)] of six rats per group. Human

lung fibroblasts IMR-90 cells were selected as positive control for TLR-2 protein levels. * $p < 0.05$ versus nonventilated, septic rats; *** $p < 0.001$ versus nonventilated, septic rats; † $p < 0.001$ versus septic animals ventilated with low V_T plus PEEP; $\tau p < 0.001$ versus septic animals ventilated with low V_T plus PEEP

Immunohistochemical localization of IRAK-3

IRAK-3 was present in both the cytoplasm and nucleus. IRAK-3 was detected in the interstitium and bronchiolar epithelium of unventilated septic lungs. Positive cytoplasmic staining for IRAK-3 was found in epithelial type II cells and interstitial macrophages surrounding the alveolus in the lungs of spontaneous breathing animals and those ventilated with low V_T . Positive staining of lung tissue with specific antibodies confirmed identification and localization of both cell types (see Fig. S1 of ESM). Positive staining for IRAK-3 was greatest in lungs ventilated with high V_T (Fig. 5).

Discussion

Our study suggests that the TLR signaling pathway plays an important role in perpetuating pulmonary inflammatory responses which are dependent on the ventilatory strategy used during sepsis-induced ALI. Our results further support the use of low V_T and moderate PEEP to minimize ventilator-induced ALI and to interfere with pro-inflammatory signaling cascades, limiting release of injurious host-derived molecules. To our knowledge, this is the first report linking the cellular response to MV with

the specific inflammatory response caused by a systemic, nonpulmonary bacterial infection, and the first to provide evidence that MV regulates TLR-4/IRAK-3/I κ B α levels in the lung in sepsis-induced ALI.

How mechanical forces are sensed by immune cells and converted into biochemical signals for intracellular signal transduction is unclear. Experimental [2–4, 27–31] and clinical [5] studies have shown that mechanical stretch can release proteins and mediators from a variety of lung cells. In addition, an imbalance of pro-inflammatory and anti-inflammatory cytokines predisposes to cellular injury and activation of secondary signaling pathways [32]. It might be possible that the changes in TLR-4 and IRAK-3 expression are increased by MV, as are many other genes [31, 33], but do not fully contribute to the augmentation of gene transcription and enhanced inflammation induced by MV in the septic lung. Many [4, 6, 33, 34] have reported the importance of the “two-hit model”: a previous pulmonary or systemic insult may sensitize lungs to injurious MV; however, injurious MV may also sensitize lungs to further pulmonary and systemic complications.

In an isolated perfused lung model in mice, Held et al. [22] found that MV with high V_T activated NF- κ B with release of cytokines in a similar manner to that of LPS. They concluded that the underlying mechanism by which overventilation activated NF- κ B was unrelated to TLR-4.

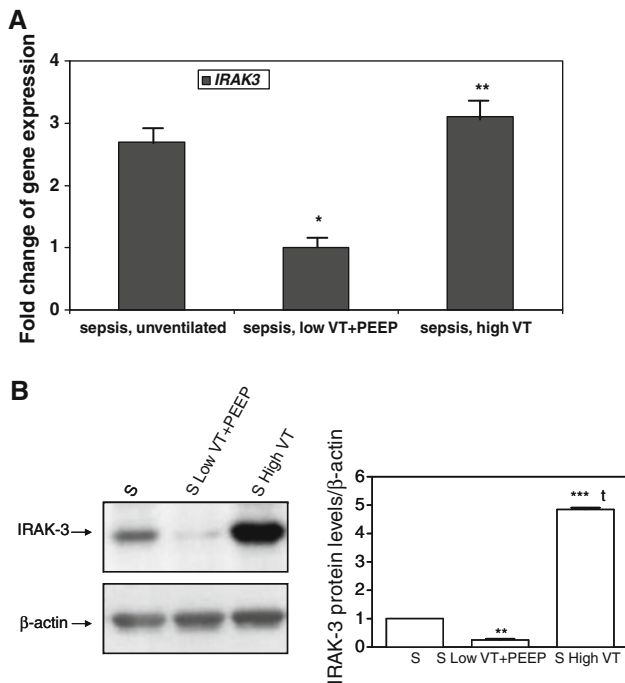


Fig. 4 **a** Fold changes in *IRAK-3* gene expression in septic lungs after 4 h of spontaneous breathing (nonventilated) or mechanical ventilation with 6 ml/kg plus 10 cmH₂O PEEP and 20 ml/kg and no PEEP. Bars represent the median fold increase compared with nonventilated animals. * $p < 0.004$ versus nonventilated, ** $p < 0.003$ versus ventilated with low V_T plus PEEP. **b** Changes of *IRAK-3* protein levels in lungs of septic rats after 4 h of mechanical ventilation with low or high V_T . Bars represent densitometric values of *IRAK-3* protein levels from all animals in each group ($n = 6$ animals per group). Data are reported as mean \pm SD ($n = 6$ independent experiments). S septic, nonventilated rats; S low V_T + PEEP septic rats ventilated with low V_T plus 10 cmH₂O PEEP; V_T tidal volume; S high V_T septic rats ventilated with high V_T . ** $p < 0.01$ versus septic, nonventilated rats; *** $p < 0.001$ versus septic, nonventilated rats; $t p < 0.001$ versus septic rats, ventilated with low V_T plus PEEP

Moriyama et al. [23] examined the inflammatory effects in rabbits of ventilation with small (5 ml/kg) or large (20 ml/kg) V_T after intratracheal LPS instillation. They postulated

that the enhanced immune response to LPS resulting from ventilation with large V_T may partially be controlled via upregulation of CD14. Previous studies have demonstrated increased NF- κ B activation in ARDS patients [35], and that NF- κ B activation in peripheral leukocytes of septic patients correlates with sepsis severity [36]. Furthermore, early alterations in neutrophil activation, particularly involving the ability to accumulate NF- κ B in the nucleus, have been associated with outcome in ALI [14]. Vaneker et al. [37] reported the effects of 4-h MV using 8 ml/kg V_T plus 4 cmH₂O PEEP in healthy and knockout TLR-2 and TLR-4 mice and found that MV of healthy mice resulted in increased expression of endogenous TLR-4 ligands in the bronchoalveolar lavage (BAL) and enhanced *TLR-4* lung gene expression associated with increased levels of TNF- α and IL-6. They found that MV increased messenger RNA (mRNA) expression of *TLR-2* in lung tissue compared with nonventilated animals. However, ventilation of TLR-2 knockout mice did not result in different cytokine levels in lung tissue homogenates and plasma compared with their wild-type ventilated controls. They also found that MV did not increase cytokines plasma levels in TLR-4 knockout mice.

The modulation of TLR signaling may be a two-edged sword. On the one hand, TLRs are essential components of the innate immune response to pathogens; on the other hand, increased TLR expression and/or signaling may contribute to disease. Activation of TLRs induces expression of hundreds of genes in macrophages. TLR-induced genes follow two distinct patterns of activation on prolonged exposure to LPS or during early stages of sepsis: genes (including pro-inflammatory cytokines) are transiently silenced, which is associated with decreased inflammation, while others (including antimicrobial proteins) are activated, as an adaptation to the innate immune response [38]. Gene reprogramming that generates silencing of pro-inflammatory genes is typified by repression of mediators that initiate both systemic and local acute inflammation. This gene silencing or repression phase may develop rapidly and occurs variably in

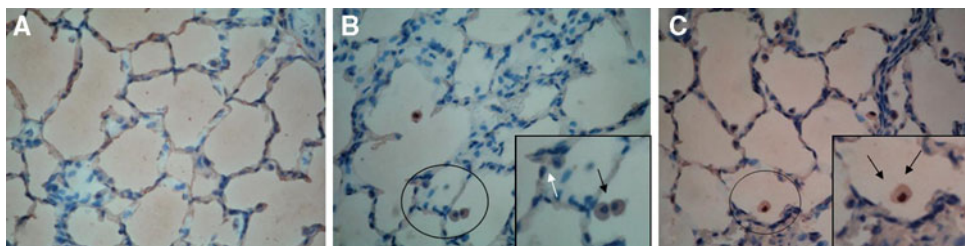


Fig. 5 Representative immunohistochemical staining for *IRAK-3* in lung tissues of various groups of septic animals. **a** Spontaneous breathing rats, **b** rats ventilated at low V_T (6 ml/kg) plus 10 cmH₂O PEEP, **c** rats ventilated at high V_T (20 ml/kg). *IRAK-3* expression is higher in lungs from septic rats ventilated at high V_T (**c**). In the inset to **b**, positive type II cells located at the corner of converging alveolar septa (white arrow), and positive macrophages

(black arrow). In inset to **c**, cytoplasmic and nuclear localization of *IRAK-3* in an interstitial macrophage. *IRAK-3* protein was visualized using 3-amino-9-ethylcarbazole as substrate (red-pink color), and nuclei were lightly counterstained using hematoxylin (blue/violet color). The insets are magnification of the indicated areas in **b** and **c**, respectively. Scale bars 40 μ m

infectious and in noninfectious clinical states of severe inflammation, such as blunt trauma or hemorrhagic shock [39–41]. Sepsis causes upregulation of IRAK-3, which counteracts the TLR-4 signaling pathway leading to expression, production, and release of inflammatory mediators. Using a CLP model of sepsis in mice, Deng et al. [42] found marked upregulation of *IRAK-3* in alveolar macrophages and modest induction of cytokine mRNA in the lungs at 12–24 h after intratracheal instillation of bacteria. The marked upregulation of *IRAK-3* by high- V_T MV in our study suggests that it could be partially responsible for mitigating the extent of inflammation and alveolar injury during the course of sepsis. By contrast, MV with low V_T plus PEEP was able to reverse the upregulation of IRAK-3, a negative TLR regulator that in immunosuppression is upregulated; and this upregulation allows downregulatory TLR signaling, since the immune system needs to strike a balance constantly between activation and inhibition to avoid harmful and inappropriate inflammatory responses [43]. Many of the pro-inflammatory mediators that appear to be involved in the pathogenesis of ALI are under the regulatory control of NF- κ B [14, 44]. In our experiments, injurious ventilation caused marked degradation of I κ B α , resulting in higher lung gene expression and systemic levels of cytokines. It appears that modulation of TLR signaling during high- V_T MV is important for maintenance of the initial LPS signal transduction that can lead to development of sepsis, multiple organ failure, and death. Studies exploring the effects of blocking the TLR-4 signaling pathway in models of ventilator-induced ALI are necessary to support this putative link. In this regard, TLRs and the IRAK proteins family might be therapeutic molecular targets which interfere with local or systemic inflammatory responses during critical illness.

Irrespective of the specific mechanism underlying IRAK-3 signaling, an interesting finding from our study is that the low- V_T group not only had lower *TNF- α* and *IRAK-3* mRNA levels than the high- V_T group, but also had similar or lower levels compared with spontaneous breathing animals. These data suggest that the low- V_T strategy may not only be less injurious but may in fact be therapeutic. We do not have a definitive explanation for this finding, but we postulate that ventilation of lungs with pre-existing damage using low V_T and moderate PEEP levels was able to attenuate sepsis-induced ALI by a number of mechanisms: (1) animals ventilated with such a “protective” ventilation strategy were better oxygenated and ventilated than spontaneously breathing animals, (2) low V_T and moderate PEEP avoids overdistension and the opening and closing of unstable lung units, and (3) in nonventilated septic animals, the failure of the alveolar epithelial barrier promotes further alveolar flooding and may increase transendothelial fluid and

protein flux [45]. Septic, spontaneously breathing animals likely developed more hypoxemia, acidosis, and hemodynamic compromise, given that four of ten spontaneously breathing animals died in the 4-h experimental period. PEEP recruits flooded alveoli and improves oxygenation by redistributing extravascular lung water from alveoli to the perivascular space [46]. In addition, protective MV can modify gene expression of important components of the extracellular matrix and accelerate remodeling and repair of damaged lung tissue [47].

We acknowledge that the present study has some limitations. First, although we have shown that MV modulated TLR-4 activation in the context of sepsis-induced ALI, we did not conclusively demonstrate that TLR pathways are involved in the increased inflammation associated with high- V_T MV because we did not examine the effect of disrupting these pathways. Second, we did not use IRAK-3-deficient animals to irrefutably demonstrate that IRAK-3 contributes to the pathogenesis of ventilator-induced ALI in the context of sepsis-induced ALI. However, studies from other investigators [42] have indicated that IRAK-3 is a selective regulator of different TLR downstream signaling processes. Third, a control group with nonseptic, healthy animals would have been useful for comparing the respective effects of MV and sepsis. We think that our data could have important clinical implications in the setting of sepsis-induced ALI. Both Altemeier et al. [33] and our group [4, 48] have reported that MV functions as a cofactor in development of ALI/ARDS and modulates the expression of inflammatory mediators in previously healthy animals or in response to sepsis or LPS. Our data also support the concept of TLR-4-targeted therapy in the context of sepsis-induced ALI. It has recently been reported in a mouse model of lethal peritonitis that anti-TLR-4 antibodies inhibited intracellular signaling, markedly reduced cytokine production, and protected mice from lethal endotoxic shock, when administered in a prophylactic and therapeutic manner [49].

In summary, our study offers a novel mechanism that could partially explain how MV modulates the innate immune response in the lung by interfering with cellular signaling pathways that are activated in response to pathogens. Collectively, these findings suggest a novel role for IRAK-3 in which it directly participates in NF- κ B-associated transcriptional events. Our findings warrant future study regarding the selectivity and specificity of innate immunity regulation.

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