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EXPERIMENTAL

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WNT/β-catenin signaling is modulated by mechanical ventilation in an experimental model of acute lung injury

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Abstract *Purpose:* The mechanisms involved in lung injury progression during acute lung injury (ALI) are still poorly understood. Because WNT/β-catenin signaling has been shown to be involved in epithelial cell injury and hyperplasia during inflammation and sepsis, we hypothesized that it would be modulated by mechanical ventilation (MV) in an experimental model of sepsis-induced ALI. *Methods:* This study was a prospective, randomized, controlled animal study performed using adult male Sprague–Dawley rats. Sepsis was induced by cecal ligation and perforation. At 18 h, surviving animals were randomized to spontaneous breathing or two strategies of MV for 4 h: low tidal volume (V_T) (6 ml/kg) plus 10 cmH_2O of positive end-expiratory pressure (PEEP) versus high (20 ml/kg) tidal volume (V_T) with zero PEEP. Histological evaluation, measurements of WNT5A, total β-catenin, and matrix metalloproteinase-7 (MMP7) protein levels by Western blot, and their immunohistochemical localization in the lungs were analyzed. *Results:* Sepsis and high-V_T MV caused lung inflammation and perivascular edema with cellular infiltrates and collagen deposition. Protein levels of WNT5A, β-catenin, and MMP7 in the lungs were increased in animals with sepsis-induced ALI. High-V_T MV was associated with higher levels of WNT5A, β-catenin, and MMP7 protein levels ($p < 0.001$), compared to healthy control animals. By contrast, low-V_T MV markedly reduced WNT5A, β-catenin, and MMP7 protein levels ($p < 0.001$). *Conclusions:* Our findings demonstrate that the WNT/β-catenin signaling pathway is modulated early during sepsis and ventilator-induced lung injury, suggesting that activation of this pathway could play an important role in both lung injury progression and repair.

Keywords Acute lung injury · WNT/β-catenin pathway · Ventilator-induced lung injury · Metalloproteinase

Introduction

Acute lung injury (ALI) is a frequent complication of sepsis, constitutes an indication for mechanical ventilation (MV), and has a significant impact on patient outcome. Resolution of the alveolar epithelial/capillary membrane damage that occurs during ALI requires a coordinated and effective tissue reconstruction program to re-establish a functional barrier [1–3]. Regeneration of alveolar epithelial cells and matrix turnover are controlled by regulatory pathways that often recapitulate the important steps in lung morphogenesis and development [4, 5]. Dysregulation of these pathways may result in amplification of the initial injury and disorderly repair, with sustained inflammation and development of pulmonary fibrosis [4–8].

There is evidence that WNT signaling plays an important role in sustained inflammation in sepsis [7, 8]. The WNT signaling pathway comprises a family of highly evolutionarily conserved secreted growth factors that activate multiple signaling pathways controlling cell proliferation, differentiation, and migration [9]. WNT molecules have been grouped as canonical and non-canonical pathway activators [7] and appear to function by binding to the family of Frizzled receptors, as well as to other transmembrane proteins [4, 7, 9, 10]. The canonical signaling blocks degradation of cytosolic β-catenin and involves the translocation of β-catenin to the nucleus and binding to nuclear transcription factors, resulting in the regulation of more than 50 mammalian genes. Non-canonical signaling is β-catenin independent; although less well defined, current evidence suggests that it modulates cell motility. WNT5A is a macrophage-derived molecule highly specific for macrophage activation and it has been implicated in pulmonary disorders [10, 11]. High serum WNT5A levels in septic patients suggest a possible role for WNT5A in severe inflammation [11]. WNT5A activates the non-canonical signaling pathway, although it can also activate or inhibit canonical signaling [12]. Target genes of the canonical β-catenin pathway include tissue matrix metalloproteinases (MMPs), which contribute to inflammation-induced tissue destruction. Pulmonary macrophages and type II epithelial cells are capable of producing MMPs, such as MMP7, a key regulator of pulmonary fibrosis [13].

Because it has been reported that WNT/β-catenin signaling is involved in epithelial cell injury [4, 11], we hypothesized that the WNT/β-catenin signaling plays an important role during ventilator-induced lung injury (VILI). This signaling pathway has not been tested in the context of VILI. To test this hypothesis, we examined the activation and localization of WNT5A, β-catenin, and MMP7 in lungs of septic animals ventilated with protective and injurious MV.

Methods

Animal preparation and experimental protocol

The experimental protocol was approved by the Hospital Universitario N.S. de Candelaria Research Committee and the Committee for the Use and Care of Animals, University La Laguna, Tenerife, Spain. Healthy, male Sprague–Dawley rats (CRIFFA, Barcelona, Spain) weighing 325–350 g ($n = 40$) were anesthetized by intraperitoneal injection of 50 mg/kg body weight ketamine hydrochloride and 2 mg/kg xylacine. Six rats were randomly selected serving as healthy, unventilated, spontaneous breathing controls. In the remaining 34 animals, sepsis was induced by cecal ligation and puncture (CLP) [14, 15]. A detailed description of this model is provided in the Electronic Supplementary Material (ESM). Eighteen hours after CLP, anesthetized surviving septic animals were randomly divided into three groups: unventilated, ventilated with protective MV, and ventilated with an injurious MV strategy. In animals allocated to MV, a cervical tracheotomy was performed using a 14-G Teflon catheter. Thereafter, animals were paralyzed with 1 mg/kg of pancuronium bromide and connected to a time-cycled, volume-limited rodent ventilator (Ugo Basile, Varese, Italy). During animal manipulation and surgical instrumentation prior to initiation of MV, oxygen saturation (SpO_2) was monitored with a pulse oximeter applied to the rat's tongue (Nellcor, Hayward, CA) and displayed continuously on a multi-parametric monitor (Philips M3561A, Holland). During animal instrumentation and transition from spontaneous breathing to MV, SpO_2 remained at least 90% in all animals.

One group of animals with healthy lungs and one group of animals with sepsis served as anesthetized, spontaneous breathing controls and were observed for 4 h. Two groups were mechanically ventilated for 4 h at FiO_2 of 0.6 using either (1) low tidal volume (V_T) (6 ml/kg) plus 10 cmH_2O of positive end-expiratory pressure (PEEP) or (2) high V_T (20 ml/kg) with zero PEEP. We kept minute ventilation constant in each group during the MV period by changing the respiratory rate. In pilot studies, we found that by maintaining minute ventilation constant, we could obtain similar $PaCO_2$ in both groups. The animals were monitored non-invasively to minimize the possibility of triggering an inflammatory response by invasive procedures. In previous pilot studies using invasive monitoring we had established that our model of CLP-induced ALI resulted in respiratory failure with hypoxemia ($PaO_2 = 60 \pm 4$ mmHg), hypercapnia (53 ± 4 mmHg), and metabolic acidosis ($pH = 7.19 \pm 0.03$) with animals breathing room air. With $FiO_2 = 0.60$, these septic animals had hemodynamic stability and adequate arterial oxygenation at the end of the 4-h observation period (PaO_2 141 ± 21 vs. 159 ± 29 mmHg, and $PaCO_2$ 43 ± 4 vs. 40 ± 3 mmHg, for the low-V_T plus PEEP and

high-V_T groups, respectively) ($n = 5$ rats/group). All animals were maintained supine on a restraining board inclined 20° from the horizontal. Rectal temperature was monitored and maintained at 36 ± 1°C with radiant heat lamps. Ventilated animals were anesthetized with ketamine/xylacine and paralyzed with pancuronium bromide by intraperitoneal injection. Peak airway pressures were continuously monitored.

Histological examination

At the end of the 4-h observation and ventilation period, a midline thoracotomy/laparotomy was performed in the first 6 surviving rats in each experimental group. In mechanically ventilated animals, blood samples for blood gases were obtained by cardiac puncture from the left ventricle of a beating heart. The abdominal vessels were transected and the hearts and lungs were removed en bloc from the thorax. The lungs were isolated, the trachea was cannulated, and the right lung was fixed by intratracheal instillation of 3 ml of 10% neutral buffered formalin. After fixation, the lungs were floated in 10% formalin for a week. Lungs were sampled in multiple areas, serially sliced from apex to base, and specimens were embedded in paraffin, then cut (3-µm thickness), stained with hematoxylin-eosin and with the Masson–Goldner trichrome technique. Slides were viewed using a Nikon Optiphot light microscope (Tokyo, Japan) and photographed with a Nikon Digital DS-5M camera (Tokyo, Japan) at ×200 magnification. See ESM for details.

Western blot analysis

Left lungs were excised, washed with saline, frozen in liquid nitrogen, and stored at −80°C for subsequent protein extraction. Lungs were homogenized and proteins were extracted by centrifugation (14,000 rpm) for 5 min at 4°C and protein concentrations in each experimental condition were determined by the Bio-Rad DC Protein Assay [16, 17]. Detection of WNT5A, total β-catenin, and MMP7 protein expression was performed in random samples by Western blotting.

Because the cyclin D1 gene is one of the target genes for the WNT/β-catenin signaling pathway and vascular endothelial growth factor (VEGF) is required for maintenance of adult lung alveolar structures, we also measured cyclin D1 and VEGF protein levels by Western blotting in the lungs of all experimental groups (details in ESM).

Immunohistochemistry for total β-catenin, WNT5A, and MMP7

Immunohistochemical stains for total β-catenin, WNT5A, and MMP7 were performed by applying a standard avidin–biotin complex (ABC) technique (details in ESM).

Statistical analysis

Values are presented as mean ± SD. Statistical analyses were performed with the Fisher exact test and paired and unpaired Student's t tests. Comparisons involving all experimental groups were performed with one-way analysis of variance. If difference were found, the Student's t test was applied. We used Bonferroni correction to address the problem of multiple comparisons. Values derived from Western blot densitometry were expressed as group mean, normalized to β-actin, and expressed by fold-changes of injured tissues versus control (healthy, unventilated) lungs, and tested with the Student's t test. Densitometry of the active form (20 kDa) of MMP7 was normalized to the inactive form (30 kDa) and then normalized to β-actin. Data management was performed using SPSS (version 15.0 for Windows). A value of $p < 0.05$ was considered significant.

Results

Outcome and pathological evaluations

All healthy animals from the control group ($n = 6$) survived the experimental period. Seven out of 34 septic rats died within the 18-h period after CLP due to sepsis. The remaining 27 septic animals were randomly allocated to the three study groups ($n = 9$ in each group). In the 4 h after removal of the cecum, 3 septic animals died in the spontaneous breathing group, none died in the low-V_T + PEEP group, and 1 septic animal ventilated with high V_T died ($p = 0.064$). Only the data from the first six surviving animals in each experimental group were analyzed. In ventilated animals, ventilatory rate was 91 ± 1 cycles/min in the low-V_T + PEEP group and 30 ± 1 in the high-V_T group. Peak inspiratory pressures never exceeded 30 cmH_2O in the high-V_T group (Table 1). Gas exchange in ventilated animals was impaired (PaO_2/FiO_2 was <250 mmHg at the end of 4 h of MV) (Table 1). The mean $PaCO_2$ was 3 mmHg lower in the high-V_T group that in the low-V_T group but this difference was not significant ($p = 0.114$).

Histopathological features of the sepsis-induced ALI animals included atelectasis, pulmonary edema, and acute inflammatory infiltrates. After 4 h of MV, septic animals ventilated with high V_T had much greater lung damage than rats ventilated with low V_T + PEEP ($p < 0.0001$), showing extensive deposition of fibrous collagen (Table 1; Fig. 1). Septic animals ventilated with low V_T + PEEP had less damage that septic, non-ventilated animals ($p < 0.001$).

Table 1 Outcome and values of ventilatory and pathological parameters after 4 h of spontaneous breathing or mechanical ventilation

Parameters	Healthy control	Septic control	Sepsis, V_T 6 ml/kg plus 10 cmH_2O PEEP	Sepsis, V_T 20 ml/kg	p value
Mortality, % (n)[a]	0 (0/6)	33.3 (3/9)	0 (0/9)	11.1 (1/9)	0.064[b]
Ventilator rate (cycles/min)	–	–	91 ± 1	30 ± 1	–
Peak airway pressure (cmH_2O)	–	–	20 ± 1	27 ± 2	0.0001
PaO_2 (mmHg)	–	–	133 ± 17	147 ± 23	0.261
$PaCO_2$ (mmHg)	–	–	42 ± 3	39 ± 3	0.114
pH	–	–	7.28 ± 0.03	7.26 ± 0.02	0.211
Histologic lung injury score	0	4.3 ± 0.5	2.5 ± 0.6	12.1 ± 2.0[b]	<0.0001[b]

Data are from 6 animals in each experimental group

[a] Seven rats died from sepsis within the 18-h period after the cecal ligation and perforation procedure

[b] When compared with all septic groups

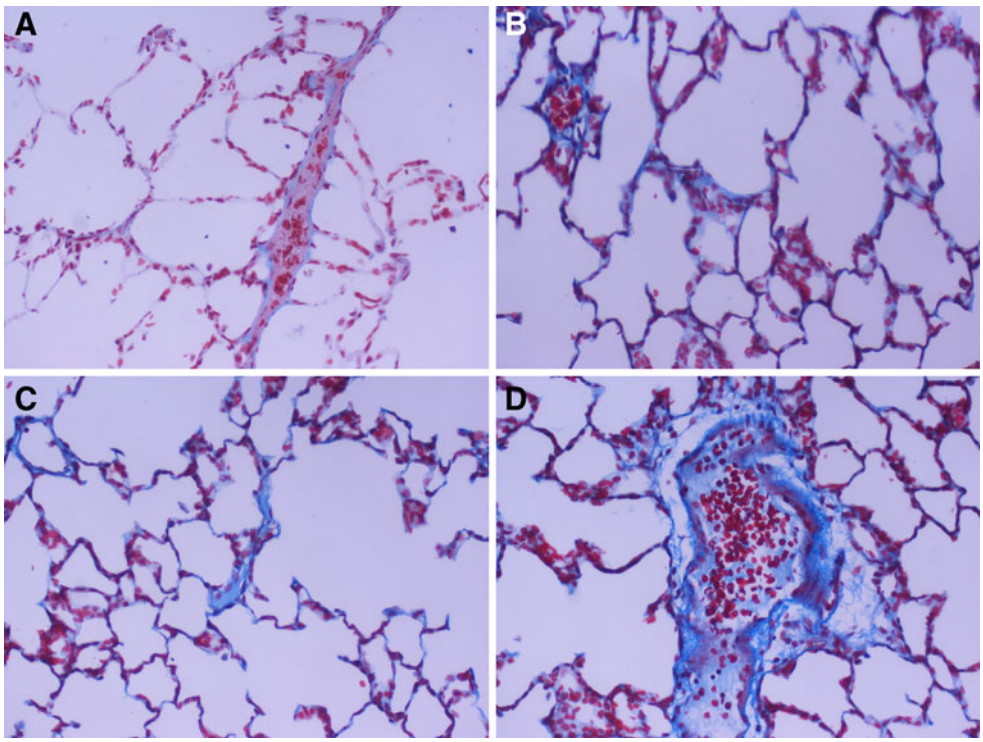

Fig. 1 Representative histopathological features of different strategies of ventilation: **a** healthy, unventilated rat lung; **b** septic lungs after 4 h of spontaneous breathing; **c** mechanical ventilation for 4 h at low V_T + PEEP; and **d** mechanical ventilation for 4 h at high V_T with zero PEEP. Animals ventilated with high V_T showed abundant pulmonary infiltrates, perivascular edema, and thickening of alveolar walls. *Blue* staining reveals deposition of fibrous collagen (Masson–Goldner, ×200 magnification)

WNT5A, total β-catenin, and MMP7 protein levels in the lungs

WNT5A protein levels increased in septic rats compared to healthy controls ($p < 0.01$, Fig. 2a). The highest WNT5A protein levels were found in septic rats ventilated with high V_T ($p < 0.001$, Fig. 2a). Ventilation with low V_T + PEEP markedly reduced WNT5A protein levels in septic rats, when compared with non-septic and septic controls ($p < 0.01$ and $p < 0.001$, respectively) (Fig. 2a).

Total β-catenin protein levels were significantly increased in septic rats (Fig. 2b). β-catenin protein levels were markedly increased in septic rats ventilated with high V_T compared to those unventilated and ventilated with low V_T + PEEP ($p < 0.001$) (Fig. 2b).

The active 20 kDa/inactive 30 kDa ratio of MMP7 protein was elevated in unventilated septic animals ($p < 0.05$) and markedly increased in septic animals ventilated with high V_T ($p < 0.001$). Ventilation with low V_T + PEEP caused a marked reduction of MMP7 protein levels ($p < 0.001$) (Fig. 3).

Immunohistochemical localization of WNT5A, total β-catenin, and MMP7

As shown in Fig. 4, WNT5A and β-catenin were sparsely detected with moderate intensity in alveolar walls and septa in the control group (1a, 1b), in non-ventilated septic animals (2a, 2b), and in those ventilated at low V_T + PEEP for

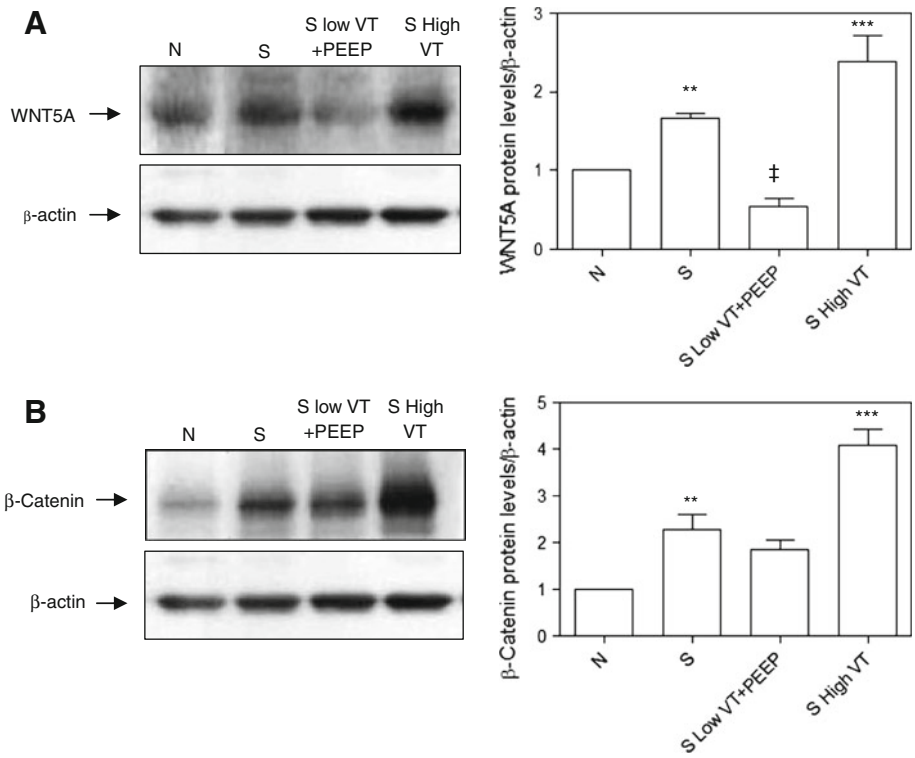

Fig. 2 Effects of mechanical ventilation for 4 h in a sepsis-induced acute lung injury model on WNT5A and total β-catenin protein levels. **a** Representative blots and bar graph showing mean densitometry values of WNT5A protein levels in rat lungs ($n = 6$ animals per group) under four experimental conditions: N = control group (non-septic, anesthetized, non-ventilated, spontaneous breathing); S = septic, anesthetized, non-ventilated, spontaneous breathing; S Low V_T = septic, ventilated with low V_T + PEEP; S High V_T = septic, ventilated with high V_T and zero PEEP. Data are reported as mean ± SD of three independent experiments. $^{**}p < 0.01$ versus healthy control, $^{***}p < 0.001$ versus septic control and septic animals ventilated with low V_T, $^{‡}p < 0.001$ versus septic rats. **b** Representative blots from individual animals and mean densitometry values of total β-catenin protein levels from all animals in each group. $^{**}p < 0.01$ versus non-septic control, $^{***}p < 0.001$ versus non-septic control and septic non-ventilated and septic animals ventilated with low V_T

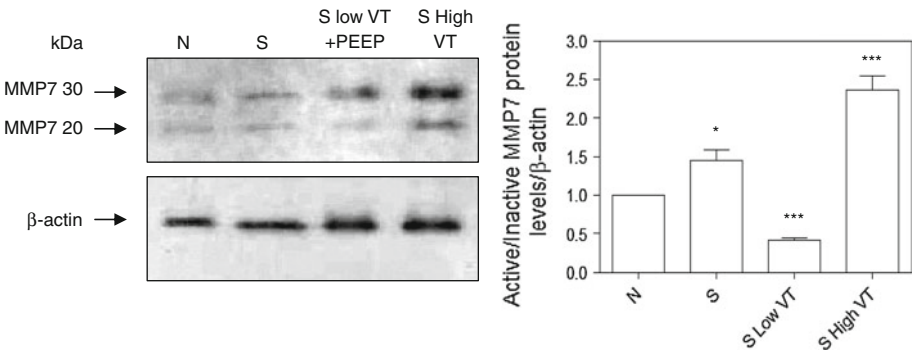

Fig. 3 Representative blots and bar graphs showing mean ± SD densitometry values of MMP7 protein levels in rat lungs ($n = 6$ animals per group) under four experimental conditions: N = control group (non-septic control, anesthetized, non-ventilated, spontaneous breathing); S = septic, anesthetized, non-ventilated, spontaneous breathing; S Low V_T = septic, ventilated for 4 h with low V_T + PEEP; S High V_T = septic, ventilated for 4 h with high V_T and zero PEEP. Data are from three independent experiments. *Blots* indicate a 30-kDa pro-MMP7 (inactive form) and a 20-kDa mature MMP7 (active form). $^{*}p < 0.05$ versus healthy rats, $^{***}p < 0.001$ versus septic control rats and septic animals ventilated with low V_T

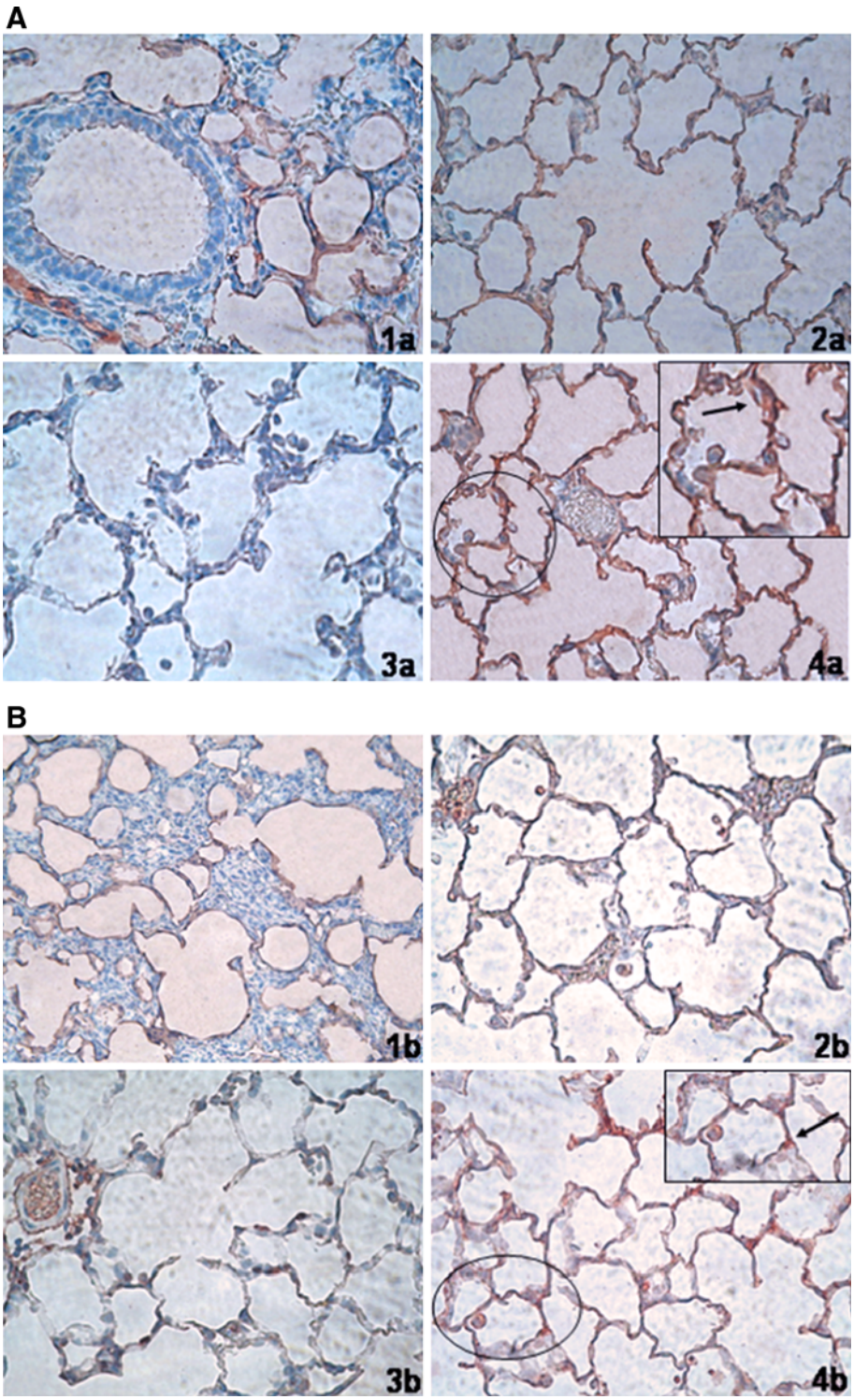

Fig. 4 Representative results of immunohistochemistry for WNT5A (*1a–4a*) and total *β*-catenin (1b–4b) in lung tissue of experimental groups. *Red-pink* color staining (3-amino-9-ethylcarbazole) indicates positive staining for WNT5A and total *β*-catenin proteins; *blue/violet* indicates nuclei counterstained with hematoxylin. Groups *1a* and *1b*, non-septic, spontaneous breathing; groups *2a* and *2b*, septic, spontaneous breathing; groups *3a* and *3b*, septic, ventilated for 4 h with low V_T plus PEEP; groups *4a* and *4b*, septic, ventilated for 4 h at high V_T with zero PEEP. WNT5A and *β*-catenin were detected with stronger intensity and more abundance in high V_T ventilated lungs. *Arrows* indicate positive alveolar epithelial cells. Images were taken with ×200 magnification. *Insets* are ×400 magnification of the areas encircled in *4a* and *4b*

4 h (3a, 3b). However, WNT5A and *β*-catenin proteins were abundantly detected with strong intensity in alveolar walls and septa in lungs ventilated with high V_T (4a, 4b).

High-V_T ventilation for 4 h significantly increased MMP7 protein staining (red-pink color), which was mainly found in alveolar walls (Fig. 5).

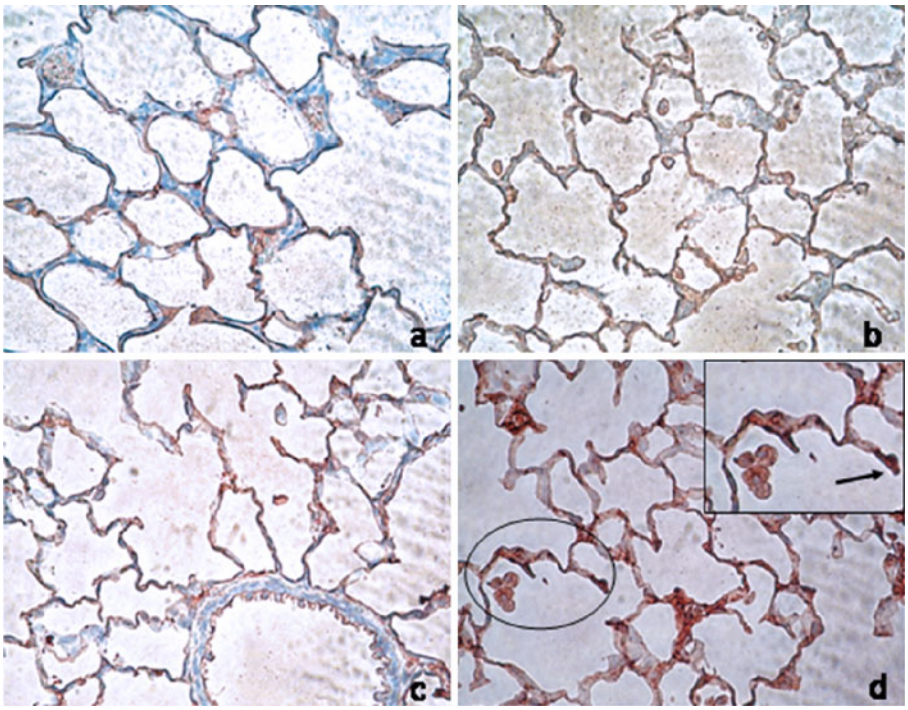

Fig. 5 Immunoreactivity of MMP7 in lung tissue of non-septic, spontaneous breathing rats (**a**); septic, spontaneous breathing (**b**); septic, ventilated with low V_T plus PEEP (**c**); and septic, ventilated for 4 h with high V_T with zero PEEP (**d**). *Red-pink* color staining indicates positive staining for MMP7 and *blue/violet* indicates nuclei counterstained with hematoxylin. MMP7 was strongly detected in alveolar walls and septa and in alveolar macrophages of animals ventilated with high V_T plus zero PEEP. *Arrow* shows positive alveolar epithelial cells, *arrowheads* indicate positive alveolar macrophages. Images were taken at ×200 magnification. *Inset* in **d** is a ×400 magnification of the encircled area

Discussion

Our study demonstrates that the WNT/β-catenin pathway is expressed and modulated in ventilated animals with sepsis-induced ALI. To our knowledge, this is the first report to examine the WNT/β-catenin pathway in an experimental two-hit model of VILI. Our main findings are as follows: (1) high-V_T MV for 4 h caused up-regulation of WNT5A protein levels; (2) this up-regulation was associated with increased total β-catenin protein levels and increased synthesis and alveolar deposition of MMP7 protein; (3) protective MV down-regulated the WNT/β-catenin signaling pathway and promoted lung recovery.

The molecular mechanisms involved in the injury and repair of airway epithelium during ALI are not well understood. One possible mechanism is via the WNT/β-catenin pathway [18–20]. The WNT family consists of a constellation of regulatory receptors, secreted and intracellular proteins, and target genes. We were interested in how MV could modulate the three molecules (WNT5A, β-catenin, MMP7) which are implicated in the process of lung repair. We chose these three molecules because activation of WNT represses β-catenin degradation, resulting in nuclear accumulation of β-catenin that leads to transcriptional activation of multiple target genes including metalloproteinases, which can then contribute to lung injury and fibrosis [21, 22]. The role of β-catenin-mediated WNT signaling is proving to be central to mechanisms of lung healing in fibrosis [18]. β-catenin signaling can stimulate tissue remodeling, cell migration, and wound closure through MMP or tissue destruction through MMP and other mediators [10]. We postulate that these signaling events are related to the reparative process directed at remodeling the alveolar architecture after sepsis and VILI.

The up-regulation of β-catenin after injurious MV suggests that modulation of the WNT/β-catenin pathway may be implicated in tissue damage and repair in response to lung injury. Tissue repair involves re-epithelization, in which injured cells are replaced by cells of the same type and normal parenchyma may be replaced by connective tissue leading to fibrosis [10]. We believe that the balance between normal and excessive expression of WNT5A is an important concept. Königshoff et al. [23] showed that WNT ligands induce lung epithelial cell proliferation, fibroblast activation, and collagen synthesis. These observations further support the importance of properly regulated WNT signaling for normal epithelial–mesenchymal interactions, and also an important process occurring during fibrotic tissue repair after injury [6].

The activation of WNT signaling after an initial injury leading to lung fibrosis, likely represents a regenerative signal of the damaged epithelium [19]. Douglas et al. [4] investigated the resolution phase after lung injury in a mouse model of oxidant-induced injury, and found increased β-catenin during the fibroproliferative phase. In our animals, there was up-regulation of total β-catenin protein levels in septic animals, with the highest levels found in animals ventilated with high V_T. These data are similar to those presented by Chilosi et al. [6] and Chang et al. [24] who found aberrant WNT/β-catenin pathway activation in idiopathic pulmonary fibrosis. Although on

the basis of their data it is not possible to define whether WNT/β-catenin activation is causative or secondary to idiopathic pulmonary fibrosis, our data suggest that this event may contribute to both impairment of epithelial repair and fibrosis. Similarly, Miyahara et al. [25] reported that total lung β-catenin was unchanged in isolated perfused mouse lungs ventilated with low V_T but was up-regulated during high-V_T ventilation. Also, during oxidant-related ALI, endothelial and epithelial damage and destruction occur, overwhelming repair mechanisms of the lung and resulting in pulmonary fibrosis [26]. Thus, the up-regulation of WNT5A and the increased nuclear β-catenin observed in our study suggests a role for WNT signaling in regulating alveolar cell and fibroblast proliferation. This event could be important for the development of ventilator-induced pulmonary fibrosis [27].

Repetitive injury and subsequent repair of epithelial type II cells, in the presence or absence of local inflammation, represent key pathogenic mechanisms in pulmonary fibrosis by promoting excessive extracellular matrix deposition [28]. Enzymes of the MMP family are involved in the breakdown of the extracellular matrix in physiological and pathological processes and are implicated during most repair processes. Except for MMP7, the MMP family is not normally expressed in healthy tissues. MMP7 is one of the five genes of interest in the context of VILI, as reported by Yerrapureddy et al. [29]. MMP found on the surface of lung epithelial cells is a key mediator of pulmonary fibrosis [30]. The enzyme encoded by the *Mmp7* gene (also called matrilysin-1) is strongly induced in injured alveolar epithelium, degrades proteoglycans, fibronectin, and elastin, and is involved in wound healing [31]. Proteoglycans in the lungs serve important functions, such as regulation of water homeostasis, maintenance of tissue structure and function, modulation of the inflammatory response, and tissue repair and remodelling [19, 32, 33]. Our findings are in line with recent observations reported by Moriondo et al. [34] who found that healthy rats ventilated for 4 h with high V_T had increased pulmonary proteoglycans and activated MMPs. *Mmp7* is a target gene of the WNT signaling pathway and has recently been identified as a key regulator of pulmonary fibrosis [23]. The progression of lung injury during VILI may involve an imbalance between the activation of MMPs and changes in the local mechanical environment of the lung. The increased up-regulation of MMP7 that we found supports a role for this enzyme in perpetuating lung inflammation and remodeling after injurious MV.

The present study has several limitations. First, although our data do not fully confirm that the WNT/β-catenin signaling pathway is involved in disrupting lung repair or in the early development of fibrosis in ALI during high-V_T ventilation, studies from other investigators [6, 7, 11, 35] have indicated that the WNT/β-catenin pathway is a selective regulator of inflammation and fibroblast proliferation and a target for anti-inflammatory and antifibrotic actions. A recent study found that administration of ICG-001 (a small molecule that specifically inhibits β-catenin transcription) concurrent with bleomycin prevented fibrosis, and late administration was able to reverse established fibrosis and significantly improved survival [35]. However, longer-term studies would be required to confirm the importance of this signaling pathway in the reparative process. Second, we did not use WNT5A-deficient animals to irrefutably demonstrate that it contributes to the pathogenesis of VILI and pulmonary fibrosis in sepsis-induced ALI. However, MMP7-deficient mice were protected from bleomycin-induced pulmonary fibrosis [13], suggesting that MMP7 may actively participate, directly or indirectly, in pulmonary fibroproliferation. Thus, our data support the concept of WNT5A- or MMP7-targeted therapy in ALI by inhibition of WNT5A expression or direct blockade of WNT5A signaling [36]. Third, a non-septic, high-V_T ventilated group may provide further supporting evidence that the WNT/β-catenin signaling pathway is involved in lung resolution following injury.

In summary, our study supports the involvement of the WNT5A/β-catenin pathway in a VILI model. We suggest that up-regulation of this pathway is involved in the pulmonary reparative processes. A greater understanding of modulators of *Wnt* gene expression and the effects of WNT proteins in sepsis and ALI will be paramount in clarifying the role of this pathway. Modulation of this pathway may represent a promising therapeutic target for attenuating or preventing the pathological consequences of ALI.

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