

# Effect of Recombinant Human Thrombomodulin on Ventilator-Induced Lung Injury in Septic Rats

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## Research Article

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# Abstract

**Background:** High tidal ventilation with inflammation causes ventilator-induced lung injury (VILI). We previously found that recombinant thrombomodulin (rTM) has a protective effect regarding non-septic VILI caused by high-tidal-volume (HV) ventilation with high oxygen levels. This study aimed to investigate the preventive effect of rTM on VILI caused by sepsis and HV ventilation.

**Methods:** A total of 46 adult male rats were subcutaneously administered either 3mg/kg of rTM or saline. Twelve hours later, the rats were underwent cecal ligation and puncture (CLP). At 2 h after this procedure, the rats were placed on a ventilator set at either low tidal volume [(LV) 6 ml/kg] or high tidal volume (HV 35 ml/kg) ventilation for another 2 h.

**Results:** After 2 h of mechanical ventilation, the PaO<sub>2</sub> was significantly lower and BALF protein was significantly higher in HV rats than in LV rats. The rTM did not improve oxygenation or BALF protein levels. Also in HV rats, lung tissue interleukin-6 and monocyte chemoattractant protein-1 mRNA levels were significantly higher in the rTM-treated rats.

**Conclusion:** rTM does not improve oxygenation in a non-DIC, CLP-pretreated, high-tidal-ventilation rat model.

## Background

High-tidal-volume ventilation causes lung damage associated with lung inflammation as well as a procoagulant and anti-fibrinolytic state (1–5), referred to as ventilator-induced lung injury (VILI) (3, 6). Although ventilation can cause VILI in the healthy lung (7), mechanical ventilation (MV) exacerbates pulmonary inflammation in an already diseased lung in the presence of lipopolysaccharides (LPSs) (8–10) and highly concentrated inhaled oxygen (11) in animal models. Mortality among patients with acute respiratory distress syndrome (12) has been reduced by low-tidal-volume (LV) ventilation, suggesting that preexisting lung damage is exacerbated by high-tidal-volume (HV) ventilation (13, 14).

Thrombomodulin is a transmembrane glycoprotein receptor for thrombin. Endothelial and soluble thrombomodulins have anticoagulant and anti-inflammatory effects via both protein C-dependent and independent pathways, by which they may prevent lung injury by directly inhibiting high-mobility group box-1 protein (HMGB-1) and LPSs (15–20). Recombinant thrombomodulin (rTM) is a novel drug approved for treating disseminated intravascular coagulation (DIC) in Japan, which might reduce in-hospital and all-cause mortality among patients with sepsis-induced DIC (21). rTM prevents LPS-induced lung injury in mice (15) and rats (22), post-pneumonectomy lung injury in mice (23), ischemia-reperfusion injury in rat liver (24), anti-cancer drug-induced liver injury in rats (25), and glomerulonephritis in rats (26), all of which display an inflammatory reaction, with or without sepsis. Previously, we showed that rTM effectively prevents lung injury induced by high-level oxygen with HV ventilation by inhibiting the production of certain interleukins (IL-1 $\alpha$ , IL-1 $\beta$ , IL-6) and macrophage inflammatory protein (MIP)-2 (11)—all of which combined represent a non-septic model.

In the clinical setting, the most frequent cause of lung injury is sepsis, and such patients are frequently subjected to mechanical ventilation. Thus, the purpose of the present study was to determine if rTM could prevent VILI in a bacterial sepsis model. We designed an experiment using the cecal ligation and puncture (CLP) model, which is more relevant to the clinical situation than LPS administration.

The purpose of this experiment was first, to prove the improvement of PaO<sub>2</sub>, second to prove the improvement of bronchoalveolar lavage fluid (BALF) protein level. We choose these because clinical definition of ARDS is established by the PaO<sub>2</sub>/FiO<sub>2</sub> ratio and BALF protein level is frequently described as a marker of lung injury. Additionally, markers to identify sepsis and DIC were measured, and cytokines and chemokines were analyzed to further clarify mechanisms of protection against lung injury.

## Methods

### Experimental Group

A total of 46 male Sprague-Dawley rats (Clea Japan, Tokyo, Japan) weighing 250–350 g were the subjects of this study. We have been used male rats from previous works. The Animal Experiments Committee of Mie University School of Medicine, Mie, Japan, approved the study protocol. The rats were injected with rTM 3 mg/kg i.p. (Asahi Kasei Pharma, Tokyo, Japan) dissolved in saline or with saline alone 12 h before the start of mechanical ventilation (MV). The rats were assigned to one of four groups as follows: (1) CLP rats (with sepsis, or Sep) pretreated with saline (TM-negative) and LV ventilation (low-tidal-volume, 6 ml/kg) [LV/Sep/TM(-); *n* = 13]; (2) CLP rats pretreated with rTM (TM-positive) and then treated with LV ventilation [LV/Sep/TM(+), *n* = 8]; (3) CLP rats pretreated with saline and then treated with HV ventilation (high-tidal-volume, 35 ml/kg) [HV/Sep/TM(-); *n* = 16]; (4) CLP rats pretreated with rTM and then treated with HV ventilation [HV/Sep/TM (+), *n* = 9]. Low and high tidal volumes were determined as described in a previous study (11). An additional set of rats that were pretreated with saline and then underwent sham operations were treated with LV ventilation for lung mRNA analysis [LV/Sham/TM(-), *n* = 4).

### CLP Method

Under pentobarbital (45 mg/kg i.p.) anesthesia, the rat's abdomen was incised 3–5 cm, and the cecum was extracted 3 h before the start of MV. For CLP rats, the cecum was ligated just above the ileocecal valve (27), and a small incision was made using electrocautery (Gemini Cautery System; Brain Science Idea Co., Tokyo, Japan) to extract feces, following which the abdomen was closed. For the sham rats, the abdomen was closed with no ligation or puncture.

### Catheterization

After the CLP procedure, the left internal carotid artery was cannulated, and Silastic tubing (0.31 mm inner diameter, 0.64 mm outer diameter) was inserted to accommodate measuring the arterial pressure

and sampling arterial blood for gas analysis (11). The mean arterial pressure was recorded using a physiological transducer and amplifier system (AP 620; Nihon Kohden, Tokyo, Japan).

## **Mechanical Ventilation**

A tracheostomy was performed just before initiating mechanical ventilation. A plastic cannula (SP-110; Natsume, Tokyo, Japan) was inserted into the trachea and ventilation was started using an SN-480-7 volume cycle ventilator (Shinano Co., Nagoya, Japan) (11). Volume controlled ventilation was applied, and the tidal volume was 6 ml/kg in low-tidal-volume ventilation group (LV) and 35 ml/kg in high-tidal-volume ventilation group (HV). To maintain arterial carbon dioxide tension at a pressure of 40–50 mmHg, the respiratory rate was adjusted to 30–100 breaths/min. (11).  $FiO_2$  was set at 0.21 (room air at sea level). PEEP was not applied.

## **Experimental Protocol**

The experimental time course was as follows. The first arterial blood gas analysis and complete blood count were performed after the CLP procedure but before starting MV (before). At 3 h after completing the CLP, tracheostomy was performed, and MV was started with a tidal volume of 6 ml/kg. Soon after the start of MV, arterial blood gas was assessed (start), and the experimental tidal volumes were assigned. MV was continued for another 2 h, with further arterial pressure and blood samples obtained every 30 min. Arterial blood gas was analyzed using a portable blood gas analyzer (i-STAT System; Brain Science Idea Co.). At the end of the experiment (i.e., 2 h after starting MV), adequate dose of pentobarbital was intravenously injected to sacrifice the animals. BALF was obtained using 2.5 mL of phosphate-buffered saline and 1.5 mL of air in two separate aliquots. Each aliquot was centrifuged (10,000 rpm, 10 min), and the cell-free supernatant was stored immediately at  $-80^{\circ}\text{C}$ . This time course was selected so it was equivalent to that of our previous experiment.

## **Biochemical analysis**

Biochemical analysis was performed using a commercial kit (total protein: BCA™ protein assay kit; Pierce Chemical Co., Rockford, IL, USA); IL-6: EIA kits; BD Biosciences Pharmingen, San Diego, CA, USA); thrombin activity and thrombin–antithrombin III complex (TAT): enzyme immunoassay kits (Cedarlane Laboratories, Hornby, ON, Canada).

## **Measurement of plasma TM**

The TM levels were determined using high-performance liquid chromatography (LC-10A; Shimadzu, Kyoto, Japan). The TM detection limit was 0.5 FU/mL.

## **cDNA preparation and real-time polymerase chain reaction**

Among the experimental groups, the first five rats and additional four sham-operation rats were chosen for further mRNA study: [LV/Sham/TM(-),  $n = 4$ ]; LV/Sep/TM(-),  $n = 5$ ]; LV/Sep/TM(+),  $n = 5$ ]; HV/Sep/TM(-),  $n = 5$ ]; HV/Sep/TM(+),  $n = 5$ ]. Lung samples were obtained to undergo real-time polymerase chain reaction (PCR). IL-6, Monocyte chemoattractant protein-1 (MCP-1), IL-10, transforming

growth factor  $\beta$  (TGF $\beta$ ), and transient receptor potential vanilloid 4 (TRPV4) mRNA levels were determined using real-time PCR. After extraction of total RNA from whole lung tissue using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), cDNA synthesis was performed using the ReverTra Ace kit (Toyobo Co., Osaka, Japan). Gene expression was measured with the StepOne Plus Real Time PCR System (Applied Biosystems, Foster City, CA, USA) using TaqMan Gene Expression Assays. PCR primers specific for IL-6 (Rn01410330), IL-10 (Rn01644839), TGF $\beta$  (Rn00572010), MCP-1 (Rn00580555), TRPV4 (Rn00576745), and  $\beta$ -actin (Rn00667869) were used. *Actin* was used as a reference gene. Amplification was performed with a StepOne Plus Real Time PCR System (Applied Biosystems). Relative quantification was performed using the comparative  $\Delta\Delta$ Ct method by normalization with  $\beta$ -actin mRNA.

## Data Analysis

Values are expressed as means  $\pm$  SE. One way-analysis of variance was used to compare groups. When significant variance was found, Scheffé's test was used to establish which groups were different. Repeated measures of the analysis of variance followed by the Bonferroni correction was used to detect intragroup changes. Values lower than were detectable were calculated as the minimal detectable limit for statistical analysis. Differences were considered significant at  $P < 0.05$ .

## Results

### PaO<sub>2</sub> and BALF Protein Concentration

PaO<sub>2</sub> was similar among the groups before and at the start of MV. At 0.5 h after the start of MV, PaO<sub>2</sub> was significantly higher in the HV/Sep/TM(-) than the LV/Sep/TM(+). At 1.5 h after the start of MV, PaO<sub>2</sub> was significantly lower in the HV/Sep/TM(+) than in the LV/Sep/TM(-). At 2 h after the start of MV, PaO<sub>2</sub> was significantly lower in the HV/Sep/TM(-) and HV/Sep/TM(+) than in the LV/Sep/TM(-), suggesting that 2 h of HV ventilation impaired oxygenation.

For intra-group changes, PaO<sub>2</sub> increased until 0.5 h from the start of MV in all groups, although the differences were not significant. PaO<sub>2</sub> had significantly decreased at 2 h in the HV ventilation groups compared with that at 0.5 h after the start of MV. There was no such decrease in the LV ventilation groups (Fig. 1a).

The BALF protein concentration was higher in the HV ventilation rats than in the LV ventilation rats, suggesting increased permeability in the HV ventilation groups. Protein concentration was higher in the HV/Sep/TM(+) than in the HV/Sep/TM(-) (Fig. 1b), although without statistical significance. Overall, these results indicate that VILI was successfully induced in the HV ventilation groups and that rTM did not improve lung permeability.

### Arterial Pressure and Lactate Levels

In all groups, the mean arterial pressures decreased significantly at 2 h compared with those before starting MV. There were no significant differences among the groups 2 h after starting MV (Fig. 2a). Lactate levels insignificantly increased in all groups at 2 h compared with that before starting MV. There were no significant differences among the groups at 2 h (Fig. 2b).

## White Blood Cell Count, Coagulation Markers

There were no significant differences in the white blood cell (WBC) counts among the LV/Sep/TM(-), LV/Sep/TM(+), and HV/Sep/TM(-) (Table 1). The WBC count in the HV/Sep/TM(+), however, was significantly lower than that in the HV/Sep/TM(-). There were no significant differences among groups regarding the blood platelet count, blood TAT, or D-dimer levels (Table 1). Although not significant, the BALF TAT level tended to be higher in the HV/Sep/TM(-) than in the LV ventilation groups. BALF TAT significantly increased in the HV/Sep/TM(+) compared with that in the LV ventilation groups (Table 1). The plasma plasmin- $\alpha$ 2- plasmin inhibitor complex was undetectable in all groups (data not shown in Table 1). These results indicate that rTM did not reduce the inflammation in septic LV-ventilated rats. HV ventilation with rTM administration in septic rats worsened the systemic inflammation and activated the bronchoalveolar level of coagulation.

Table 1  
Blood cell counts and coagulation data 2 hours from start of mechanical ventilation

Group	WBC (*10 <sup>2</sup> / $\mu$ L) (Blood)	Plt (*10 <sup>4</sup> / $\mu$ L) (Blood)	TAT (ng/mL) (Plasma)	TAT (ng/mL) (BALF)	D-dimer ( $\mu$ g/mL) (Plasma)
LV/Sep/TM(-)	30.7 $\pm$ 8.2 (n = 12)	83.5 $\pm$ 16.8 (n = 12)	15.2 $\pm$ 9.7 (n = 6)	< 0.1 (n = 8)	0.04 $\pm$ 0.01 (n = 6)
LV/Sep/TM(+)	33.0 $\pm$ 4.1 (n = 6)	90.8 $\pm$ 15.5 (n = 6)	< 0.1 (n = 6)	< 0.1 (n = 6)	0.07 $\pm$ 0.04 (n = 6)
HV/Sep/TM(-)	40.8 $\pm$ 11.8 (n = 16)	78.0 $\pm$ 21.4 (n = 16)	1.75 $\pm$ 1.2 (n = 4)	0.48 $\pm$ 0.11 (n = 11)	0.05 $\pm$ 0.02 (n = 4)
HV/Sep/TM(+)	26.1 $\pm$ 10.8 (n = 7) *	83.1 $\pm$ 7.2 (n = 7)	0.28 $\pm$ 0.07 (n = 5)	0.76 $\pm$ 0.24 (n = 7) #	0.04 $\pm$ 0.01 (n = 5)
LV, low tidal volume (6 mL/kg); HV, high tidal volume (35 mL/kg); Sep, sepsis.; WBC, white blood cell; Plt, platelet; TAT, thrombin anti-thrombin complex					
*p < 0.05 vs. HV/Sep/TM(-). #<0.05 vs. LV/Sep/TM(-) and LV/Sep/TM(+).					

## BALF and Plasma IL-6 Levels

BALF IL-6 was significantly higher in the HV/Sep/TM(+) than in the LV ventilation groups, although the difference in increase was insignificant between HV/Sep/TM(+) and HV/Sep/TM(-), suggesting that the

combination of HV ventilation and rTM administration increased the BALF IL-6 (Fig. 2c). Plasma IL-6 levels were not significantly different among the groups, but there was a tendency that the rTM-treated groups had higher IL-6 levels than the non-treated group (Fig. 2d).

## Plasma and BALF TM Levels

The plasma rTM level was higher in the rTM-treated groups than in the rTM non-treated groups (Fig. 2f). Notably, the BALF TM in the HV ventilation groups was markedly increased, whereas that in the LV ventilation groups was not. These data support the finding that lung permeability was enhanced by HV ventilation (Fig. 2e).

## Lung mRNA Levels

To confirm the accentuated inflammatory response in the HV ventilation groups, we measured mRNA levels in the lungs (Fig. 3). The IL-6, MCP-1, and IL-10 mRNAs were significantly increased in the HV/Sep/TM(-) and HV/Sep/TM(+) compared with those in the sham-operation group. Notably, IL-6 and MCP-1 were significantly increased in the HV/Sep/TM(+) compared with that in the HV/Sep/TM(-). IL-10 was also increased by administration of rTM, although the difference did not reach statistical significance. TGF $\beta$ , an anti-inflammatory cytokine, was significantly decreased in the HV/Sep/TM(-) and HV/Sep/TM(+) compared with that in the sham-operation rats. Because activation of TRPV-4 induces lung vascular permeability, we measured TRPV4 mRNA and found that these levels were not significantly different among groups.

## Microscopy of BALF

BALF was microscopically analyzed in each group. Although there were no significant differences in the number of neutrophils/macrophages among groups (data not shown), we found bacterial infiltrates in BALF in the HV ventilation groups. A representative specimen is shown in Fig. 4.

## Discussion

Our results showed that we have successfully produced a lung injury model using CLP and HV ventilation that could be compared with the rats exposed to LV ventilation. However, in opposition to our hypothesis, rTM did not alleviate the lung injury, as shown by the PaO<sub>2</sub> and BALF protein levels. Moreover, although most clinical findings are consistent with rTM worsening lung injury of septic rats, the results were not statistically significant.

## Model of lung injury

CLP is a traditional model for inducing bacterial sepsis, although an earlier study showed that the CLP itself did not cause lung injury (28). Two invasions are thought to be needed to induce a VILI—hence the “two-hit theory.” There are data suggesting CLP plus HV ventilation causes VILI (29). We, however, found no oxygenation deterioration in their VILI model.

We successfully produced a lung injury model by adding HV ventilation to CLP rats, which was confirmed by the decreased PaO<sub>2</sub> level and increased protein concentration in BALF. Following the initial increase in PaO<sub>2</sub> in all groups, the PaO<sub>2</sub> did not decrease in the LV-ventilation groups but decreased significantly in the HV-ventilation groups, suggesting that HV ventilation causes lung injury. The reason that PaO<sub>2</sub> was initially increased was probably due to overstretching of the lung. HV overstretches more than LV and this caused initial higher oxygenation but resulted in higher lung injury. Lung IL-6 and MCP-1 mRNA levels increased more in the CLP groups than in the sham-operation rats, indicating that our model exhibited a sepsis-associated inflammatory response. Platelet counts and the plasma TAT and D-dimer levels were similar among the rTM non-treated groups, showing that the rats had not developed DIC. Thus, rTM was administered to septic rats that did not have overt DIC.

The concentration of BALF is normalized according to its fluid (saline) volume. The BALF protein increase and bacterial infiltration of alveoli in the HV ventilation groups indicate that mechanical stretching during MV exacerbated lung inflammation in the CLP rats. The BALF TAT concentration was significantly more increased in HV ventilation groups than in the LV ventilation groups, suggesting that HV ventilation activated coagulation in the alveoli in this group, which is also one of the characteristics of the VILI lung.

TRPV4 is a stretch-activated cation channel, which was originally identified as the sodium channel in *Drosophila*. Recently, it was found in lung endothelial cells, epithelial cells, and alveolar macrophages. TRPV4 is activated by membrane stretching, and it causes cell spreading of alveolar macrophages. Activation of these macrophages is known to cause VILI by increasing pro-inflammatory cytokines (4). Because TRPV4 knockdown mice or administration of TRPV4 receptor antagonist prevents the development of VILI (32, 33), we measured lung TRPV4 mRNA levels and found no differences among the groups. As TRPV4 mRNA expression was not increased more in the HV ventilation groups than in the LV ventilation groups, TRPV4's function, not expression alone, might cause VILI.

## Controversy Concerning rTM

Reports of the effect of rTM on the septic model have been inconsistent among studies. For example, rTM administration blocked LPS-induced acute lung injury in one study (34), whereas it improved survival in mice lacking the lectin-like domain of rTM (35).

In our experiment, rTM did not alleviate the lung injury. This is confirmed by the PaO<sub>2</sub> level and BALF protein leakage. There was a significant increase in BALF IL-6 in rTM-treated, HV-ventilated rats, compared with the LV-ventilated groups, while plasma IL-6 was not significantly different among groups suggesting that induction of lung local inflammation was more sensitively occurred than plasma inflammation.

BALF TAT levels were unexpectedly significantly higher in the HV/Sep/TM(+) group than in the HV/Sep/TM(-) group. We speculated that this result might also show that rTM could not alleviate lung injury in the present study. The effect of rTM on TAT generation can be projected as follows. Plasma level thrombin generation is suppressed by rTM administration in LV ventilation groups. Administration of rTM

in the HV ventilation group accelerated the destruction of the lung wall and induced high-level local inflammation in addition to vascular permeability due to the systemic inflammation. This situation resulted in a greatly increased level of TAT in lung but not enough to be inactivated by the infiltrated rTM.

We offer three possible explanations for why rTM was not effective in the current VILI model. First, the timing of the administration may have been too early. With our experimental protocol, rTM was administered before inducing sepsis and DIC. Our results may indicate that too-early administration may have suppressed immunologically necessary trap formation. Recently rTM was shown to inhibit neutrophil extracellular trap (NET) formation (36). NET formation is known to have an important role in the host's defense against bacterial infection, but it may also induce hypercoagulability. However, we have chosen this timing because our previous experiment showed that plasma rTM level were elevated after 9 h and remained elevated for 48 h after rTM injection (37). Thus, plasma level rTM was appropriate when septic VILI has been made.

Second, our protocol was based on a bacterial infection-caused VILI model that was not treated with antibiotics. rTM has an anti-inflammatory effect by increasing anti-inflammatory cytokines (e.g., IL-1 $\beta$ , TGF $\beta$ ) in an LPS model. In the present study, TGF $\beta$  mRNA was decreased in the HV/Sep groups and was not increased by rTM administration. These data suggest that, in the presence of live bacteria, anti-inflammatory cytokines are suppressed, and rTM administration does not induce an anti-inflammatory response.

Third, the intensity of the disease was not identical among groups. CLP is a traditional method for inducing bacterial sepsis. However, the quantity of feces that spreads into the abdomen might not be equal among the subjects, and the bacteria species responsible for the infection in each rat are ultimately unknown. This situation, however, is equivalent to intestinal perforation in the clinical setting. Even if there were some diversity in the intensity of the bacterial infections, we successfully showed statistical differences in the impact of HV ventilation.

There are several limitations in this study. One is the mechanism of rTM improved oxygenation in hyperoxic VILI but not in this experiment was not clear. Infection, DIC, severity and timing are possible explanations but not sure. Further research is needed. Second, the number of the animals were small. We tried to minimize the number of animals but might be too small to show the statistical analysis. Increased number of the animals should be considered for further research.

## Conclusion

rTM pretreatment did not prevent lung injury in a septic, non-DIC, hypoxic VILI rat model. Further research is needed for establishing the appropriate condition of administering rTM to achieve lung injury prevention.

## List Of Abbreviations

VILI ventilator-induced lung injury  
rTM recombinant thrombomodulin  
LV low tidal volume (ventilation)  
HV high tidal volume (ventilation)  
BALF bronchoalveolar lavage fluid  
MV mechanical ventilation  
LPS lipopolysaccharide  
HMGB-1 high-mobility group box-1  
DIC disseminated intravascular coagulation  
MIP macrophage inflammatory protein  
CLP cecal ligation and puncture  
TAT thrombin–antithrombin III complex  
PCR polymerase chain reaction  
MCP-1 monocyte chemoattractant protein 1  
TRPV4 transient receptor potential vanilloid 4  
NET neutrophil extracellular trap

## **Declarations**

### ***Ethics approval and consent to participate***

This study was approved by the Mie University Animal experiment committee. [Approval number 26-2]

All methods were carried out both ARRIVE guideline for the study and in accordance with relevant guidelines and regulations.

### ***Consent for publication***

Not applicable.

### ***Availability of data and materials***

The datasets generated and/or analysed during the current study are available in the figshare, [[https://figshare.com/articles/dataset/Effect\\_of\\_Recombinant\\_Human\\_Thrombomodulin\\_on\\_Ventilator-Induced\\_Lung\\_Injury\\_in\\_Septic\\_Rats\\_Dataset/13351976](https://figshare.com/articles/dataset/Effect_of_Recombinant_Human_Thrombomodulin_on_Ventilator-Induced_Lung_Injury_in_Septic_Rats_Dataset/13351976)]

### ***Competing interests***

The authors declare that they have no competing interests

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### ***Authors' contributions***

YI and ZE drafted the manuscript and performed the main experiments. HS, MK, and JM helped the animal experiment and advised statistical analysis. HI supervised the experiments. KS supervised the use of rTM. KM supervised the manuscript writing.

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## Figures

Fig 1.

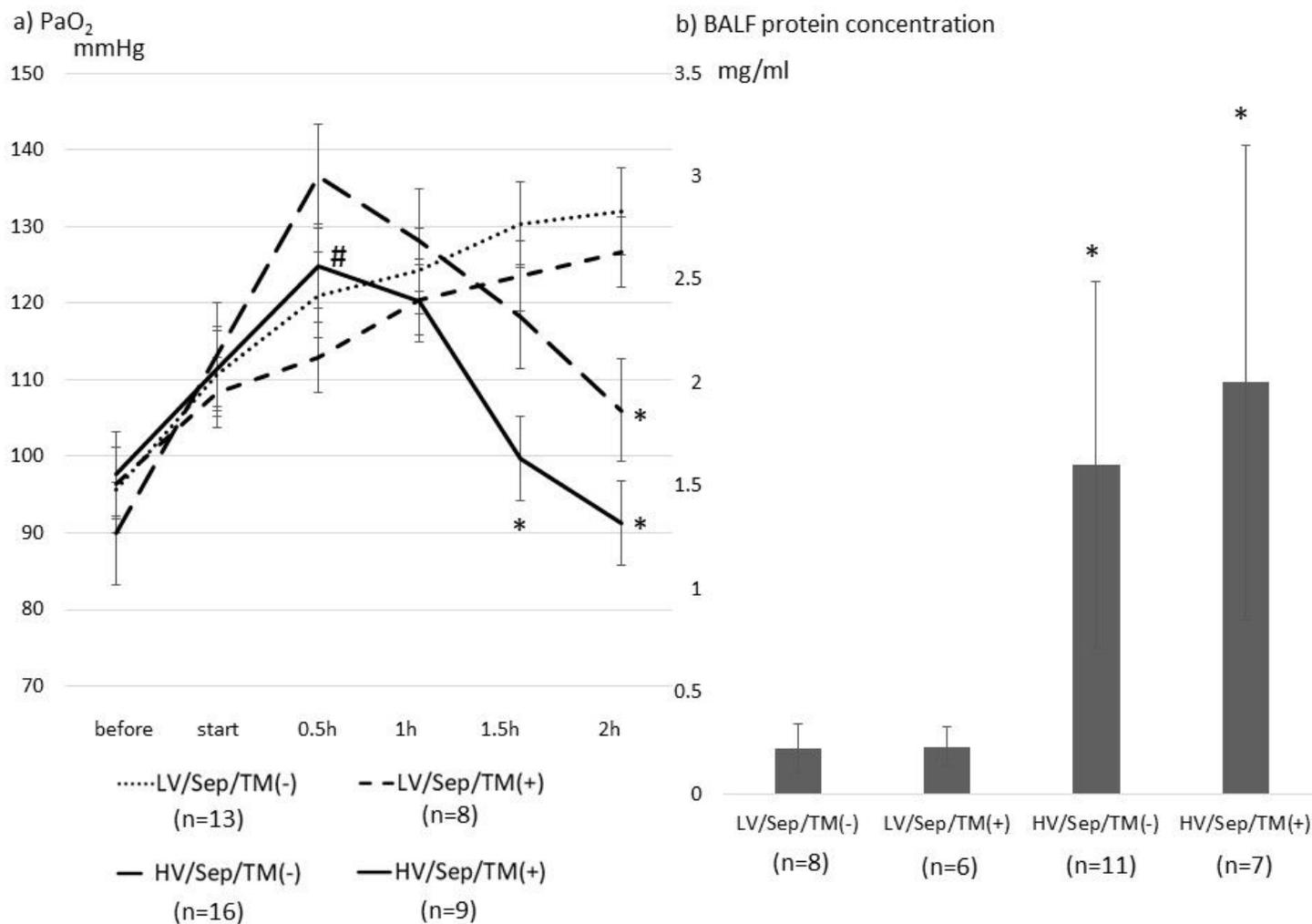
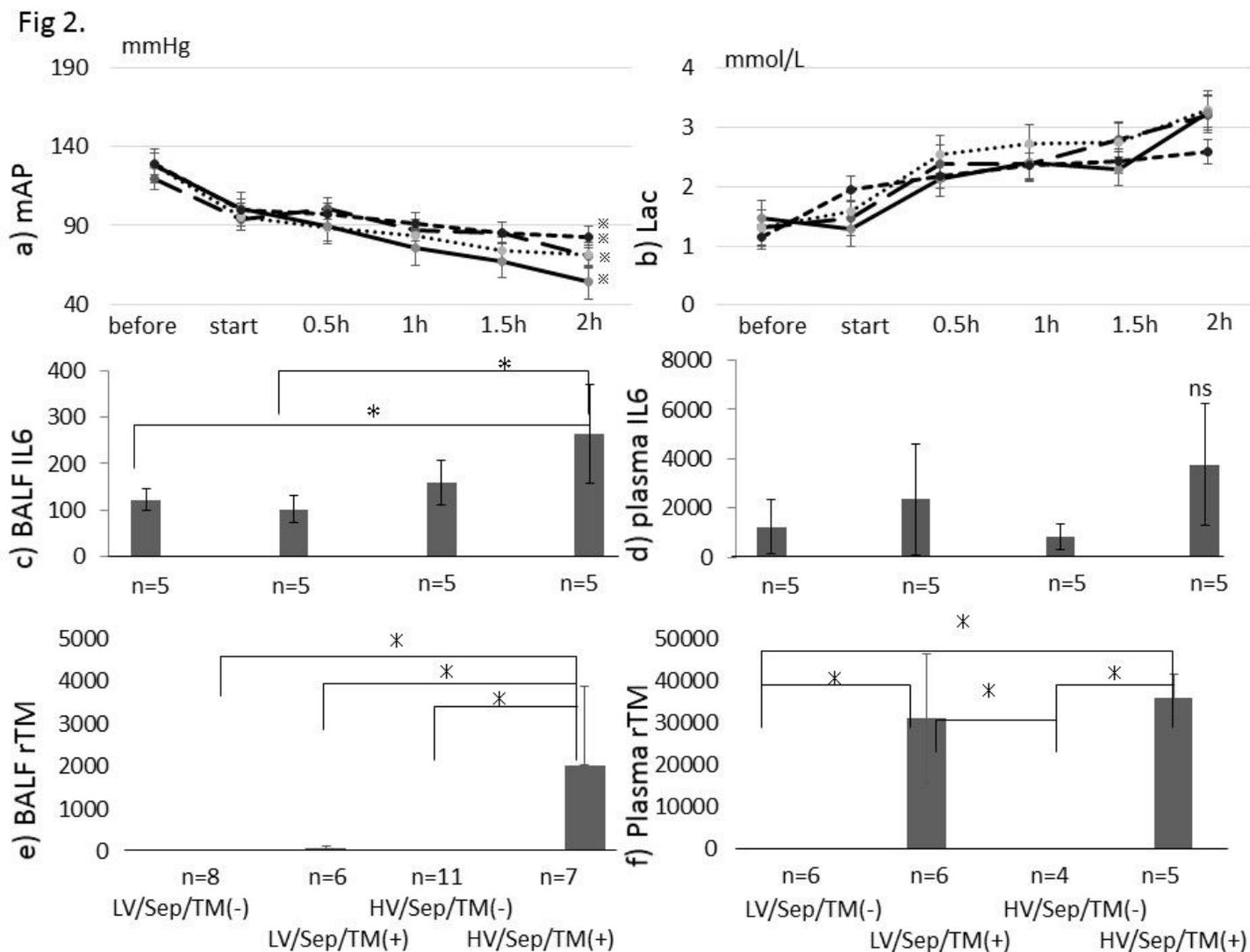


Figure 1

a, Time course of arterial oxygen pressure (PaO<sub>2</sub>). LV/Sep/TM(-) = CLP rats pretreated with saline and then treated with low tidal volume (LV) ventilation (n=13). LV/Sep/TM(+) = CLP rats pretreated with thrombomodulin (TM) and then treated with LV ventilation (n=8). HV/Sep/TM(-) = CLP rats pretreated with saline and then treated with HV ventilation (n=16). HV/Sep/TM(+) = CLP rats pretreated with TM and then treated with HV ventilation (n=9). b, Protein concentration in bronchoalveolar lavage fluid (BALF). HV ventilation groups had higher BALF protein concentrations than LV ventilation groups. CLP, cecal ligation and puncture; LV, low tidal volume (6 mL/kg); HV, high tidal volume (35 mL/kg); Sep, sepsis. \*p<0.05 vs. LV/Sep/TM(-). #<0.05 vs. LV/Sep/TM(+). Bars = means ± SE.



**Figure 2**

Time courses for the mean arterial pressure (a) and blood lactate level (b). a, Mean arterial pressures were similar among the groups at each time point. They were significantly lower at 2 h than before starting mechanical ventilation ( $\ast p < 0.05$ ). b, Blood lactate levels continued to increase during the treatment course. There were no statistical differences among the groups. c, Measurements in bronchoalveolar lavage fluid (BALF). IL-6 level was significantly higher in the HV/Sep/TM(+) group than in the LV/Sep/TM(-) and LV/Sep/TM(+) groups. d, Plasma IL-6 levels. There were no significant differences among groups. e, BALF rTM concentration was significantly higher in the HV/Sep/TM(+) group than in the LV/Sep/TM(+) group, suggesting increased permeability during HT ventilation because the molecular weight of TM is 64,000, which is less than that of albumin. f, Plasma rTM concentrations were significantly higher in the rTM-treated groups than in the non-treated groups. mAP, mean arterial pressure; Lac, lactate; MV, mechanical ventilation; IL6, interleukin-6; rTM = recombinant thrombomodulin. See Figure 1 for other abbreviations.  $\ast p < 0.05$  for each pair

Fig 3.

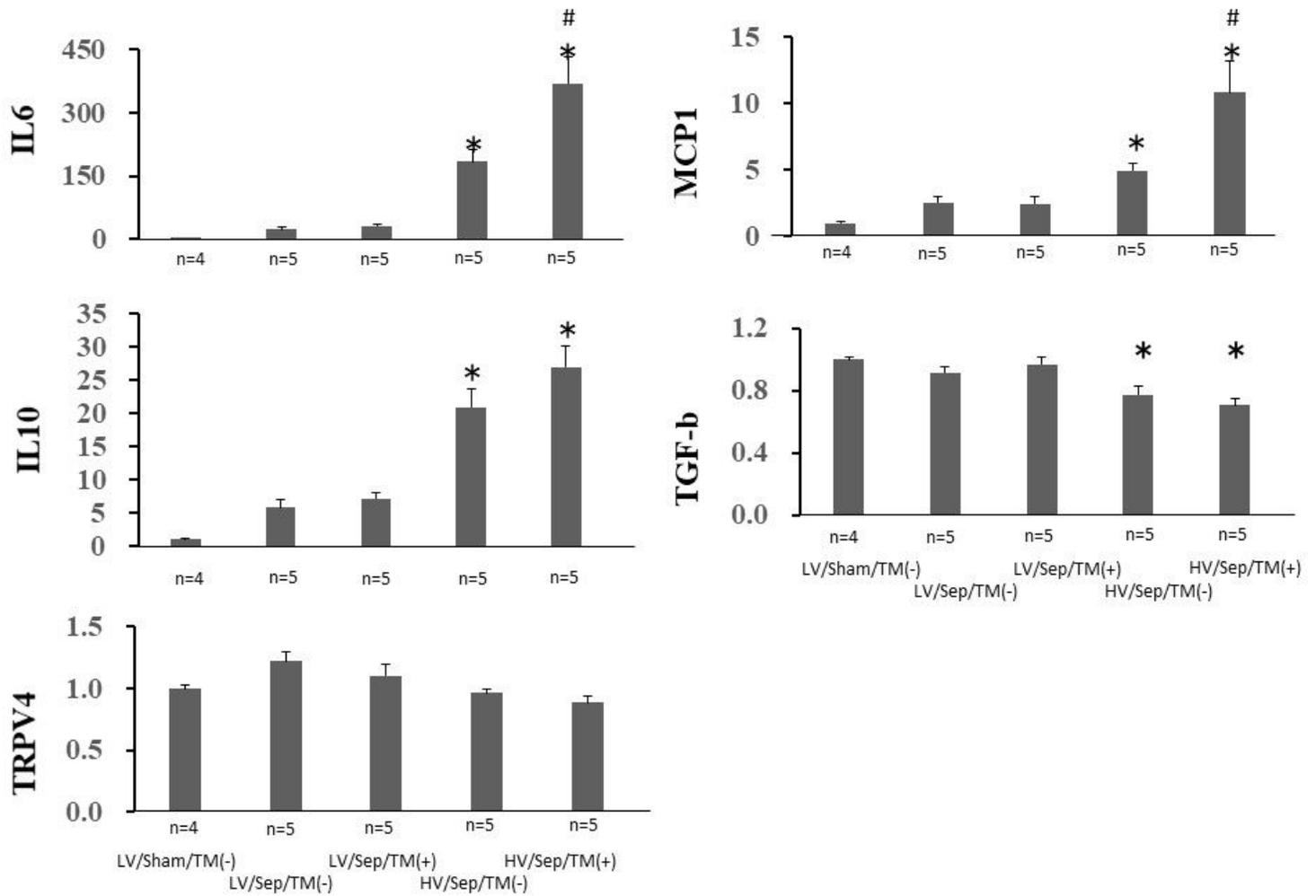
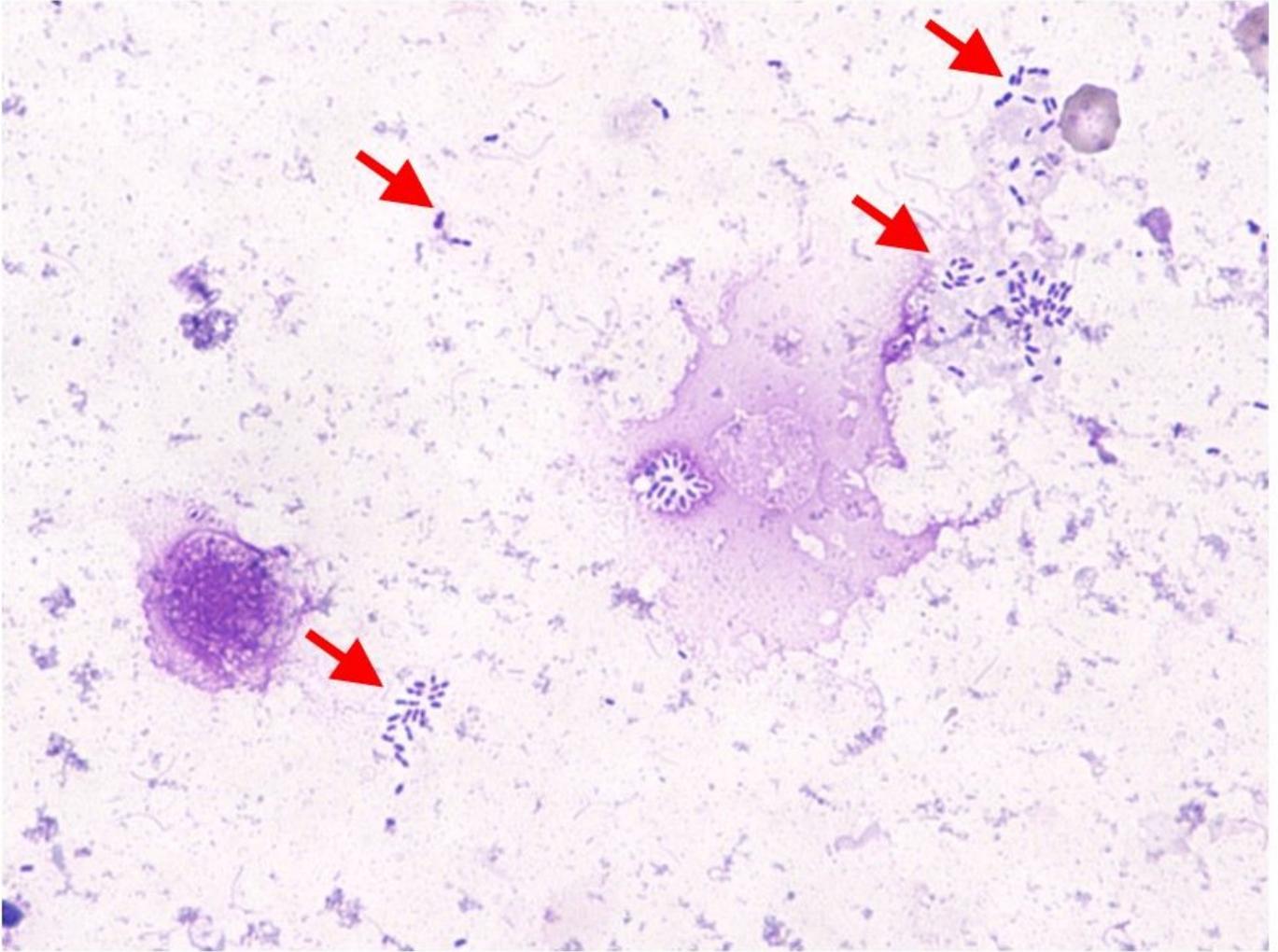


Figure 3

Lung messenger RNA (mRNA) levels of IL-6, monocyte chemotactic protein-1 (MCP-1), IL-10, transforming growth factor  $\beta$  (TGF- $\beta$ ), and transient receptor potential vanilloid 4 (TRPV4). \* $p$ <0.05 compared with sham-operated rats. # $p$ <0.05 compared with the HV/Sep/TM(-) group. See Figure 2 for other abbreviations

Fig 4.



**Figure 4**

Representative microscopic findings in BALF. Red arrows indicate bacterial infiltration in the HV/Sep/TM(-) and HV/TM(+) groups. (Gram stain,  $\times 1200$ )