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Mechanical ventilation aggravates transfusion-related acute lung injury induced by MHC-I class antibodies

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Abstract *Purpose:* Transfusion-related acute lung injury (TRALI) occurs more often in critically ill patients than in a general hospital population, possibly due to the presence of underlying inflammatory conditions that may prime pulmonary neutrophils. Mechanical ventilation may be a risk factor for developing TRALI. We examined the influence of mechanical ventilation (MV) on the development of TRALI, combining a murine MV model causing ventilator-induced lung injury with a model of antibody-induced TRALI. *Methods:* BALB/c mice ($n = 84$) were ventilated for 5 h with low (7.5 ml/kg) or high (15 ml/kg) tidal volume, a positive end-expiratory pressure of 2 cm H₂O and a fraction

of inspired oxygen of 50%. After 3 h of MV, TRALI was induced by infusion of MHC-I antibodies (4.5 mg/kg); controls received vehicle. Non-ventilated animals receiving vehicle, isotype or MHC-I antibodies served as additional controls. *Results:* All animals receiving MHC-I antibodies developed TRALI within 2 h. In mice in which TRALI was induced, MV with low tidal volumes aggravated pulmonary injury, as evidenced by an increase in neutrophil influx, pulmonary and systemic levels of cytokines and lung histopathological changes compared to unventilated controls. The use of high tidal volume ventilation resulted in a further increase in protein leakage and pulmonary edema.

Conclusions: Mechanical ventilation (MV) synergistically augmented lung injury during TRALI, which was even further enhanced by the use of injurious ventilator settings. Results suggest that MV may be a risk factor for the onset of TRALI and may aggravate the course of disease.

Keywords Acute lung injury · MHC-I · Antibodies · TRALI · VILI

Introduction

Transfusion-related acute lung injury (TRALI) is the leading cause of transfusion-related morbidity and mortality [10, 24]. Traditionally, TRALI is viewed as a rare complication of blood transfusion. However, this may not hold true for critically ill patients. An accumulating number of studies report a high incidence of TRALI in the critically ill patient population [3, 8, 20, 27, 29, 31–33], with incidences ranging from 5.1–8% per transfused patient, compared to 0.02–0.16% per transfused patient in the general hospital population [8, 20, 27, 31–33]. These differences may be explained by the proposed “two hit” mechanism of TRALI [21]. The “first hit” is the underlying condition of the patient which results in priming of the pulmonary neutrophils (e.g. sepsis, pneumonia). The “second hit” is caused by the transfusion of antibodies directed against the recipient’s antigens or biologically active lipids which accumulate during storage. As critically ill patients frequently suffer from an underlying condition, this patient group may be more susceptible to developing TRALI.

Although mechanical ventilation (MV) may be an inevitable procedure, MV can induce or aggravate lung injury, referred to as ventilator induced lung injury (VILI). Low tidal volume ventilation reduces mortality in patients with acute lung injury [1], indicating that ventilator settings influence pulmonary injury. Notably, ventilation with low tidal volumes also induces neutrophil-mediated lung injury [4, 34, 36]. Therefore, it can be hypothesized that MV may prime pulmonary neutrophils, rendering the lungs more susceptible to a TRALI reaction. In an observational study, as many as 33% of mechanically ventilated critically ill patients developed lung injury within 48 h after transfusion [7]. Also, transfusion and large tidal volumes appear to have a synergistic effect on the development of ALI [6]. However, no direct evidence exists as to whether MV may be a priming factor for the onset of TRALI, or whether it may aggravate the course of a TRALI reaction.

Although it is usually stated that the prognosis of TRALI is good [19], recent studies show that TRALI is associated with increased morbidity and mortality in the critically ill [3, 8, 20, 27]. Therefore, it is important to elucidate the pathogenesis of TRALI in this patient group. To investigate whether MV affects the development and course of a TRALI reaction, we adopted a well-established model of VILI, in which TRALI was induced by infusion of MHC-I antibodies [9, 17, 36].

Methods

The study was approved by the Animal Care and Use Committee of the Academic Medical Center at the

University of Amsterdam, Amsterdam, The Netherlands. Animal procedures were carried out in compliance with Institutional Standards for Human Care and Use of Laboratory Animals. In the online-supplement, the “Method” section is described in more detail.

MHC-I mAb

A hybridoma (34-1-2S) was purchased from the American Type Culture Collection that produces mAb against H2K^d (IgG_{2a}, κ), which have previously been shown to induce TRALI in an animal model [17]. As isotype matched antibodies we used an IgG_{2a}, κ producing hybridoma (CRL-1908), from the American Type Culture Collection.

Mice

Experiments were performed with healthy male BALB/c mice ($n = 84$) (Charles River, Someren, the Netherlands), aged 8–10 weeks and weights 19–25 g, randomly assigned to 7 groups ($n = 12$ per group) (Fig. 1, online-supplement). Three groups (infusion of PBS, infusion of isotype antibody, infusion of MHC-I class antibody) served as non-ventilated controls and were killed after 2 h. The other animals were mechanically ventilated with two different strategies for 5 h and received either PBS infusion or MHC-I class infusion after 3 h of ventilation.

Mechanical ventilation strategies and monitoring

Animals were anesthetized as previously described [35, 36]. Mice were placed in a supine position, connected to a ventilator (Servo 900 C, Siemens, Sweden) and pressure-controlled ventilated with either an inspiratory pressure of 10 cm H₂O (resulting in lung-protective $V_T \sim 7.5$ mL/kg; low tidal) or an inspiratory pressure of 18 cm H₂O (resulting in injurious $V_T \sim 15$ mL/kg; high tidal). Respiratory rate was set at 110 breaths/min and 70 breaths/min with low tidal and high tidal, respectively. After 3 h, the jugular vein was isolated. Using a 30-gauge sterile needle, venous blood was aspirated from the jugular vein to verify intravascular placement of the needle and to remove a sample of blood (~ 200 μ l). Mice were given an i.v. volume-matched injection (150–250 μ l) of either MHC I mAb (4.5 mg/kg) or PBS. Systolic blood pressure and heart rate were non-invasively monitored using a murine tail-cuff system (AD Instruments, Spence, Germany) and recorded on a data acquisition system (PowerLab/4SP, ADInstruments).

Study groups and sampling

Non-ventilated control mice were spontaneously breathing and were killed after 2 h. LV_T -mice and HV_T -mice were mechanically ventilated for 5 h and then killed. Subsequently, in 6 animals, bronchoalveolar lavage fluid (BALF) was obtained from the right lung and cell counts were determined using a hemacytometer (Beckman Coulter, Fullerton, CA). Differential counts were done on cytospin preparations stained with a modified Giemsa stain, Diff-Quick (Dade Behring AG, Düringen, Switzerland). Supernatant was stored at -80°C for total protein level and cytokine measurement. The left lung was used to determine the wet to dry ratio. Another 6 mice were used for blood gas analysis from blood sampled from the carotid artery and for histopathology of the lungs (fixed in 4% formalin and embedded in paraffin).

Assays

Blood gas analysis was done in a Rapidlab 865 blood gas analyzer (Bayer, Mijdrecht, the Netherlands). The other blood samples were centrifuged and the supernatants were aliquoted and frozen at -20°C . Total protein levels in BALF were determined using a Bradford Protein Assay Kit (OZ Biosciences, Marseille, France) according to the manufacturer's instructions with bovine serum albumin as standard. Cytokine and chemokine levels were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Tumor necrosis factor (TNF)- α , interleukin (IL)-6, macrophage inflammatory protein (MIP)-2 and keratinocyte-derived chemokine (KC) assays were obtained from R&D Systems (Abingdon, UK).

Statistical analysis

All data in the results are expressed as mean \pm sem or median \pm interquartile range, where appropriate. To detect differences between groups, paired T test, Dunnett's method or Mann-Whitney U test was used when appropriate. A p value of <0.05 was considered statistically significant. All statistical analyses were carried out using SPSS 12.0.2 (SPSS, Chicago, IL).

Results

Hemodynamic and ventilatory monitoring

In non-ventilated control animals, respiratory rate did not change after infusion of vehicle or isotype control antibody (Fig. 2, online-supplement). All control animals

survived the 2 h. In contrast, mice infused with MHC-I antibodies showed respiratory distress and tachypnea shortly after infusion. Survival after 2 h in non-ventilated animals with TRALI was reduced to 60%.

All ventilated animals survived the 5 h of MV. Blood gas analysis showed that animals achieved adequate gas exchange. Systolic arterial pressure and heart rate remained stable in all animals throughout the experiment.

Infusion of MHC-I antibodies results in TRALI in non-ventilated animals

MHC-I antibodies induced TRALI in non-ventilated animals, as indicated by an increase in respiratory rate (Fig. 2, online-supplement, $p < 0.01$), accompanied by an increase in pulmonary wet-to-dry ratio (Fig. 1, $p < 0.01$), pulmonary neutrophil influx (Tables 1, 2, $p < 0.05$) and protein leakage of the lungs (Fig. 2, $p < 0.01$) compared to vehicle and isotype controls. Histopathological changes showed an increase in pulmonary neutrophil sequestration compared to controls (Table 2; Fig. 3).

Pulmonary levels of IL-6 and KC were increased in non-ventilated mice challenged with MHC-I infusion compared to controls (Fig. 4, $p < 0.01$). The antibodies also induced a systemic inflammatory reaction, increasing plasma levels of IL-6, KC and MIP-2 compared to controls ($p < 0.01$), whereas TNF did not change. (Fig. 5).

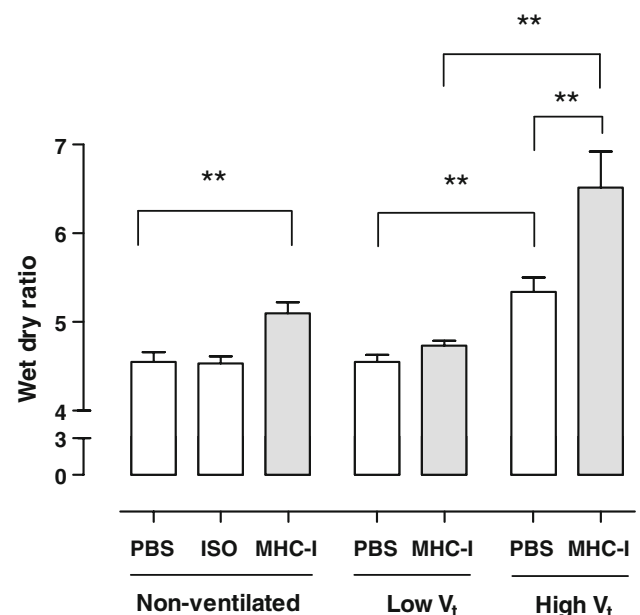


Fig. 1 Wet to dry ratio of the lungs. Non-ventilated animals receiving PBS, isotype control antibody or MHC-I antibody. Low V_T (mice ventilated for 5 h with a tidal of 7.5 ml/kg) and High V_T (mice ventilated for 5 h with a tidal of 15 ml/kg) received PBS or MHC-I antibody infusion. * $p < 0.01$

Table 1 Arterial blood gas analysis and hemodynamic measurements

	PBS infusion		MHC-I infusion	
	Low V_T	High V_T	Low V_T	High V_T
Arterial blood gas				
pH	7.53 (0.1)	7.53 (0.1)	7.48 (0.2)	7.54 (0.1)
PaCO ₂ (mmHg)	29.1 [20.2 to 36.6]	24.4 [20.4 to 30.4]	24.0 [23.3 to 39.0]	25.5 [19.5 to 29.3]
PaO ₂ (mmHg)	158 [128 to 216]	136 [95 to 169]	133 [66 to 224]	149 [77 to 212]
HCO ₃ ⁻ (mmol/l)	22.8 (3.4)	23.3 (1.4)	20.9 (1.1)	20.8 (2.7)
BE	1.8 [0.6 to 4.1]	0.4 [-0.5 to 1.2]	2.1 [-0.8 to 5.3]	2.2 [-3.9 to 5.4]
Systolic BP				
Start MV	120 (20.6)	143 (18.5)	132 (21.5)	136 (19.3)
Pre-infusion	108 (20.3)	106 (27.5)	97 (21.0)	84 (24.0)
Post-infusion	88 (17.8)	88 (16.8)	93 (31.8)	92 (13.9)
Heart rate				
Start MV	350 (27.7)	379 (30.1)	354 (43.6)	391 (26.9)
Pre-infusion	363 (53.9)	387 (54.9)	385 (44.2)	364 (46.8)
Post-infusion	350 (38.5)	339 (47.8)	378 (81.1)	370 (57.8)

Data are mean (SD) or median [IQR]; Low V_T = mice ventilated for 5 h with a tidal of 7.5 ml/kg; High V_T = mice ventilated for 5 h with a tidal of 15 ml/kg. $n = 6$ per group. PaCO₂ = partial pressure of arterial carbon dioxide; PaO₂ = partial pressure of arterial oxygen
BE base excess, BP blood pressure, MV mechanical ventilation

Table 2 Cell counts in bronchoalveolar lavage fluid and histopathological examination of lung tissue

	Total cells ($\times 10^4$ /ml BALF)	Neutrophil ($\times 10^4$ /ml BALF)	Lung injury score
Non-ventilated			
Control PBS	81 (48–132)	0 (0–0.3)	0 (0–0.5)
Control isotype	30 (15–77)	0 (0–0.4)	0 (0–0.5)
Control MHC-I	158 (64–313)*	8.9 (4.7–16.6)**	1.0 (1–2.5)*
Ventilated			
PBS Low V_T	136 (37–174)	1.3 (0.2–17.3)	1.0 (0–1.5)
PBS High V_T	120 (43–227)	11.3 (8.3–45.1)**	1.0 (1–3.0)*
MHC-I Low V_T	274 (72–457)*	10.1 (6.0–105.1)**	2.5 (1–5.5)
MHC-I High V_T	296 (137–363)*	27.4 (16.1–40.4)**	3.0 (3–4.5)*

Low V_T = mice ventilated for 5 h with a tidal of 7.5 ml/kg; High V_T = mice ventilated for 5 h with a tidal of 15 ml/kg. $n = 6$ per group

* $p < 0.05$ Control PBS versus Control MHC-I, MHC-I Low V_T and MHC-I High V_T

** $p < 0.01$ Control PBS and Control isotype versus Control MHC-I, PBS High V_T , MHC-I Low V_T and MHC-I High V_T

Mechanical ventilation induces lung injury in previously healthy lungs

Mechanical ventilation (MV) with high tidal volumes, but not with low tidal volumes, resulted in an increased wet-to-dry ratio, accompanied by an increased neutrophil influx and protein leakage in the BALF compared to non-ventilated controls ($p < 0.01$) (Figs. 1, 2; Table 2) as well as a higher histopathology score (Table 2; Fig. 3, $p < 0.05$).

Mechanical ventilation (MV) with high tidal volumes, but not with low tidal volumes, resulted in increased pulmonary levels of IL-6 and KC compared to non-ventilated controls (Fig. 4, $p < 0.01$). MV with high tidal volumes also induced a systemic inflammatory reaction, increasing plasma level of IL-6, KC, MIP-2 and TNF compared to non-ventilated controls (Fig. 5, $p < 0.01$). MV with low tidal volumes resulted only in increased plasma levels of IL-6 compared to non-ventilated controls.

Mechanical ventilation with protective tidal volumes aggravates lung injury induced by MHC-I antibodies

We investigated the effect of combining MV with protective ventilation settings with the TRALI model. MV with low tidal volumes in animals challenged with MHC-I antibodies resulted in an increased pulmonary neutrophil influx compared to non-ventilated animals challenged with MHC-I antibodies, together with a higher lung injury score (Table 2, $p < 0.05$; Fig. 3), but did not aggravate pulmonary protein leakage (Fig. 2) nor pulmonary wet-to-dry ratio (Fig. 1). MV with low tidal volume increased pulmonary levels of KC in mice challenged with MHC-I antibody infusion compared to non-ventilated animals challenged with the MHC-I antibody (Fig. 4, $p < 0.01$), with a non-significant increase in pulmonary levels of IL-6. MV with low tidal volume also aggravated the systemic inflammatory reaction after MHC-I antibody infusion, resulting in increased plasma levels of KC, IL-6

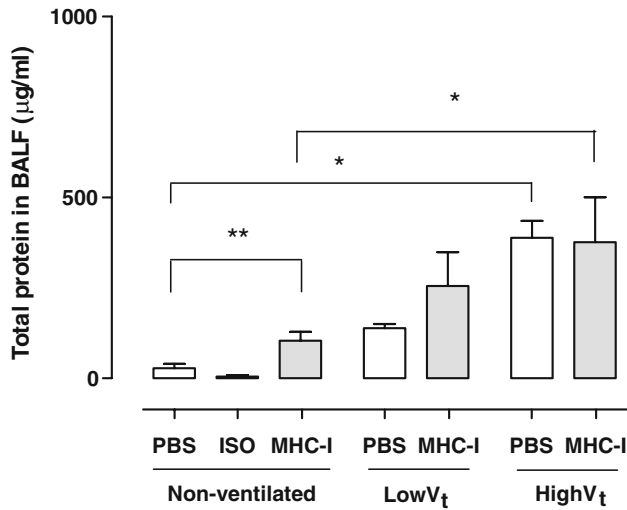


Fig. 2 Total protein leakage in bronchoalveolar lavage fluid (BALF). Non-ventilated animals receiving PBS, isotype control antibody or MHC-I antibody. Low V_T (mice ventilated for 5 h with a tidal of 7.5 ml/kg) and High V_T (mice ventilated for 5 h with a tidal of 15 ml/kg) received PBS or MHC-I antibody infusion. * $p < 0.05$, ** $p < 0.01$

and MIP-2 compared to non-ventilated animals challenged with MHC-I antibody infusion (Fig. 5, $p < 0.05$).

Mechanical ventilation with injurious tidal volume ventilation further aggravates lung injury induced by MHC-I antibodies

Next, to determine whether injury induced by mechanical ventilation contributes to the course of a TRALI reaction, the effect of injurious ventilation settings was studied in the TRALI model. MV with high tidal volumes induced an increase in wet-to-dry ratio in animals challenged with MHC-I antibodies compared to MV with low tidal volumes and non-ventilated controls challenged with MHC-I antibodies (Fig. 1, $p < 0.01$). Also, MV with high tidal volumes in animals challenged with MHC-I antibodies increased neutrophil influx (Table 2, $p < 0.05$), protein leakage in the BALF (Fig. 2, $p < 0.01$) and histopathology score (Table 2, $p < 0.01$) compared to non-ventilated animals challenged with MHC-I antibodies. Although not reaching statistical significance, protein leakage and neutrophil influx in the BALF was higher in the animals

Fig. 3 Transfusion of MHC-I antibodies aggravates ventilator induced lung injury. Histologic sections of hematoxylin and eosin stained mice lungs at 100× magnification. Non-ventilated animals receiving PBS, or MHC-I antibody. Low V_T (mice ventilated for 5 h with a tidal of 7.5 ml/kg) and High V_T (mice ventilated for 5 h with a tidal of 15 ml/kg) received PBS or MHC-I antibody infusion **a** PBS non-ventilated; **b** MHC-I non-ventilated; **c** PBS and Low V_T ; **d** PBS and High V_T ; **e** MHC-I and Low V_T ; **f** MHC-I and High V_T . Normal vasculature (**a**). Neutrophils sequestered in the vasculature (arrow) (**b–f**). Increased pulmonary edema and neutrophil extravasation (**d–f**)

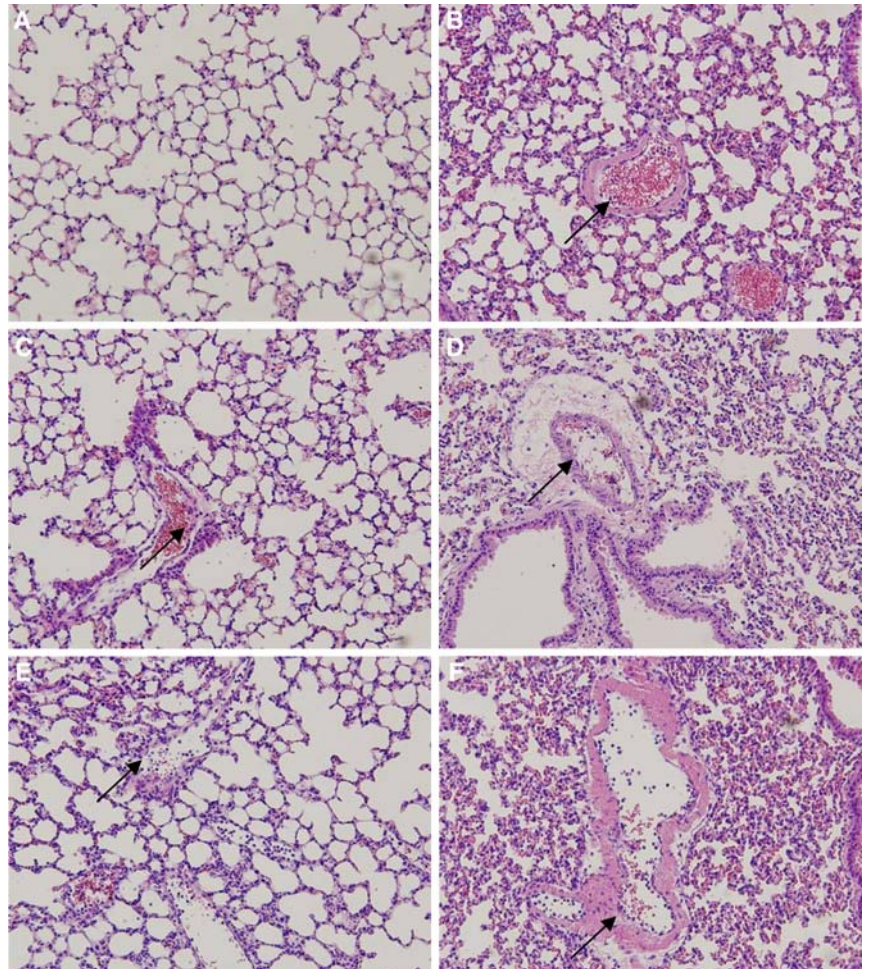


Fig. 4 Interleukin (IL)-6 and keratinocyte-derived chemokine (KC) concentrations in the bronchoalveolar lavage fluid (BALF). Non-ventilated animals receiving PBS, isotype control antibody or MHC-I antibody. Low V_T (mice ventilated for 5 h with a tidal of 7.5 ml/kg) and High V_T (mice ventilated for 5 h with a tidal of 15 ml/kg) received PBS or MHC-I antibody infusion. * $p < 0.05$ and ** $p < 0.01$

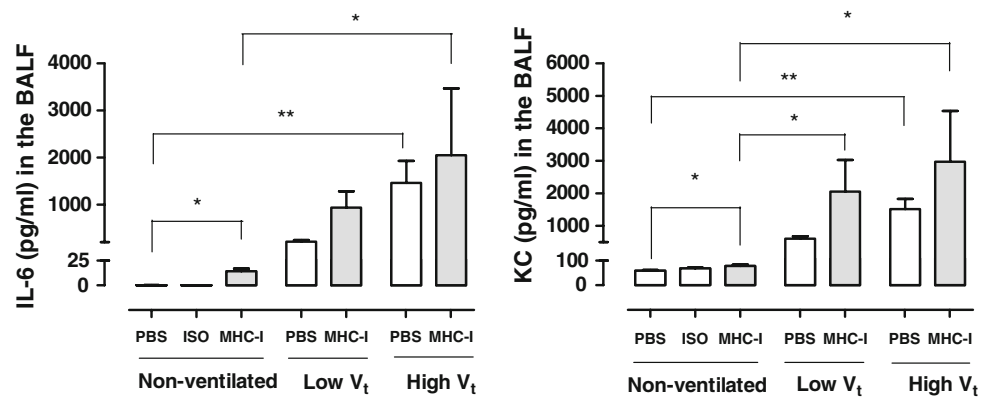
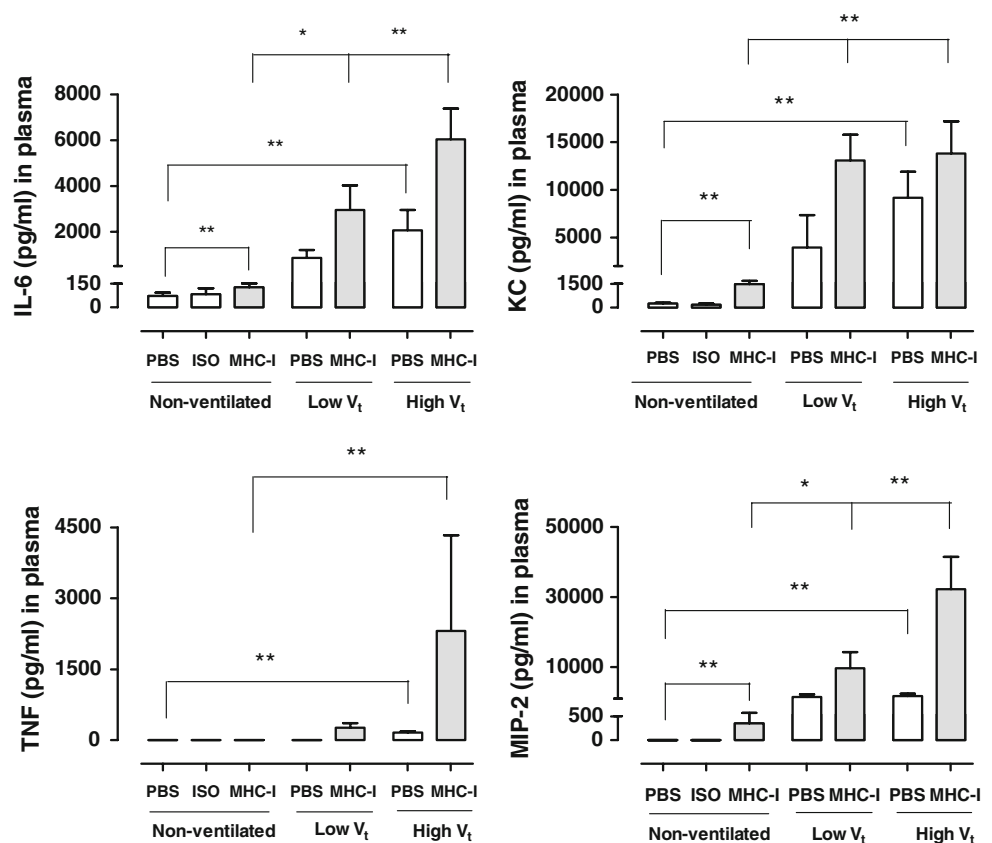


Fig. 5 Interleukin (IL)-6, keratinocyte-derived chemokine (KC), tumor necrosis factor (TNF) and MIP-2 concentrations in the plasma. Non-ventilated animals receiving PBS, isotype control antibody or MHC-I antibody. Low V_T (mice ventilated for 5 h with a tidal of 7.5 ml/kg) and High V_T (mice ventilated for 5 h with a tidal of 15 ml/kg) received PBS or MHC-I antibody infusion. * $p < 0.05$ and ** $p < 0.01$



receiving MV with high tidal volumes and MHC-I antibodies compared to MV with low tidal volumes and MHC-I antibodies.

Mechanical ventilation (MV) with high tidal volumes increased pulmonary levels of IL-6 (Fig. 4, $p < 0.05$) and KC (Fig. 4, $p < 0.001$) compared to non-ventilated controls challenged with MHC-I antibodies. MV with high tidal volume also aggravated the systemic inflammatory

reaction after MHC-I antibody infusion, increasing plasma levels of IL-6, KC and TNF compared to non-ventilated animals after MHC-I antibody infusion (Fig. 5, $p < 0.01$). Although not reaching statistical significance, pulmonary levels of IL-6 and KC and systemic levels of IL-6, MIP-2 and TNF were higher in the animals receiving MV with high tidal volumes and MHC-I antibodies compared to MV with low tidal volumes and MHC-I antibodies.

Discussion

We describe a model of antibody-mediated TRALI in a clinically relevant model of mechanical ventilation. MV synergistically augmented lung injury during TRALI, which was even further enhanced by the use of high tidal volumes. These findings support the concept that MV aggravates the pulmonary and systemic course of a TRALI reaction. We postulate that MV may serve as a priming factor, thereby rendering critically ill patients susceptible for a TRALI reaction after receiving a blood transfusion.

In this study, MV synergistically worsened histopathology, pulmonary edema, neutrophil influx and pulmonary and systemic inflammation in MHC-I antibody challenged animals, even with the use of ‘protective’ ventilator settings that did not induce lung injury. In line with this, mechanical stress induced by MV is characterized by a pro-inflammatory response. Such a response may be present even in protective ventilator settings, i.e. with settings that do not cause overt lung injury [26, 36]. Neutrophils recruited to the pulmonary compartment by the ventilator have been found to show evidence of priming [25], resembling a “first hit” in TRALI models. Thereby, during MV, pulmonary neutrophils may be more susceptible to the detrimental effects of the antibodies, resulting in activation after transfusion and the clinical symptoms of TRALI. In line with these findings, we found that MV with low tidal volumes augmented injury inflicted by TRALI antibodies compared to unventilated animals. In particular, neutrophil-influx was enhanced, as were mediators which are released by neutrophils such as pulmonary and systemic inflammation. Mechanical ventilation may predispose patients to TRALI, which may account, at least in part, for the high incidence of TRALI among the critically ill [8, 20, 27].

Mechanical ventilation (MV) with injurious settings causing VILI and antibody-induced TRALI synergistically induced lung injury in this study, suggestive of a “two hit” phenomenon in which MV is the “first hit” and the antibodies are the “second hit”. The “two hit” phenomenon in TRALI has been described after infusion of biological response modifiers that have accumulated during storage of cell-containing blood products, resulting in TRALI in primed lungs. Transfusion of the supernatant of stored blood products caused lung injury after priming with a “first hit” of endotoxin (LPS), but not in the absence of a “first hit” [12, 22, 23]. However, the “two hit” phenomenon has infrequently been described in immune-mediated TRALI [12, 16]. An explanation may be a lack of case reports. The original case description of TRALI involved patients developing acute respiratory failure after transfusion of a plasma product, in whom donor antibodies against leukocyte antigens in the recipient have been linked with the TRALI symptoms. Since

then, reports of TRALI cases predominantly describe these “classic” antibody-mediated TRALI symptoms, in which other ALI risk factors are absent [5, 13–15, 18]. In line with our results suggesting that a “two hit” phenomenon may also be present in immune-mediated TRALI, a recent previous experiment showed that antibodies could induce a TRALI reaction in the presence, but not in the absence, of LPS as a priming factor [16].

Our results underscore the relevance of low tidal volume ventilation. Although low tidal volume is now strongly recommended [1], it is still not widely implemented in ALI patients [11]. Our study demonstrates that high tidal ventilation may prime the lungs, thereby lowering the threshold to develop or worsen TRALI. This suggests that the application of low tidal volumes in patients exposed to the risk of a blood transfusion is rational. Another clinical implication pertains to the reporting of TRALI to the Blood Bank. Our results suggest that antibodies in combination with a priming factor may increase lung injury to a clinically significant degree. However, medical disciplines involved in diagnosing and reporting of TRALI consider an inflammatory condition prior to the transfusion a reason to withhold from reporting a suspected TRALI-case [30]. Our results may increase the awareness of critical care specialists, haematologists and transfusion medicine experts that TRALI may also occur in the presence of another ALI risk factor. Results also underline the importance of reporting a suspected TRALI case to the blood bank for analysis of neutrophil and leukocyte antibodies, in order to exclude implicated donors in an effort to prevent future TRALI reactions.

Plasma from multiparous donors is associated with the onset of TRALI, as these donors have been sensitized during labour, resulting in a higher incidence of the presence of leukocyte and/or neutrophil antibodies compared to plasma from male donors. From our results it can be speculated that transfusion with male only plasma may reduce the amount of lung injury in patients with a pro-inflammatory state, such as critically ill patients. In line with this, excluding female donors for high volume plasma components in the UK and The Netherlands reduced the onset of TRALI in several critically ill patient populations [2, 28, 37]. However, deferring women from plasma donation did not completely prevent the onset of TRALI. Therefore, a restrictive transfusion policy remains mandatory. Further studies are needed to identify the effect of male only plasma and restrictive transfusion guidelines of high plasma volume blood products in the critically ill on the occurrence of respiratory complications.

This study has several limitations. First, we used only a component of the blood product, which does not reflect the clinical situation. Second, not all endpoints of lung injury showed a significant difference between the vehicle and MHC-I high tidal ventilation group. This may be due to a type II error, using only 6 animals per group.

However, the lung injury endpoints which did not reach statistical significance all showed a clear trend towards a difference.

Conclusion

We developed a clinically relevant model of combining mechanical ventilation and TRALI, in which mechanical

ventilation served as a “first hit”, synergistically aggravating lung injury during TRALI, an effect that was augmented by the use of lung injurious mechanical ventilation settings. Our results suggest that in patients at risk for receiving a blood transfusion, even in the absence of lung injury, protective low tidal ventilation is a rational approach.

Conflict of interest statement None.

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