Ventilator-induced lung injury leads to loss of alveolar and systemic compartmentalization of tumor necrosis factor-α

**Abstract** Objectives: To determine the effect on compartmentalization of the tumor necrosis factor (TNF-α) response in the lung and systemically after ventilation with high peak inspiratory pressure with and without positive end-expiratory pressure (PEEP).

Design and setting: Prospective, randomized, animal study in an experimental laboratory of a university.

Subjects and interventions: 85 male Sprague-Dawley rats. Lipopolysaccharide was given intratracheally or intraperitoneally to stimulate TNF-α production; control animals received a similar amount of saline. Animals were subsequently ventilated for 20 min in a pressure control mode with peak inspiratory pressure/PEEP ratio of either 45/0 or 45/10 (frequency 30 bpm, I/E ratio 1:2, FIO₂ = 1).

Measurements and results: Blood gas tension and arterial pressures were recorded at 1, 10, and 20 min after start of mechanical ventilation. After killing of the animals pressure-volume curves were recorded, and bronchoalveolar lavage (BAL) was performed for assessment of protein content and the small/large surfactant aggregate ratio. TNF-α was determined in serum and BAL. TNF-α levels were significantly increased after lipopolysaccharide stimulation; furthermore ventilation without PEEP resulted in a significant shift of TNF-α to the nonstimated compartment as opposed to ventilation with a PEEP level of 10 cmH₂O.

Conclusions: Ventilation strategies which are known to induce ventilation-induced lung injury (VILI) disturb the compartmentalization of the early cytokines response in the lung and systemically. Furthermore, the loss of compartmentalization is a two-way disturbance, with cytokines shifting from the vascular side to the alveolar side and vice versa. A ventilation strategy (PEEP level of 10 cmH₂O) which prevents VILI significantly diminished this shift in cytokines.

**Key words** Lung injury · Mechanical ventilation · Cytokines · Multiple-organ failure · Compartmentalization · Ventilation associated pneumonia

**Introduction**

The leading cause of death in acute respiratory distress syndrome is multiorgan failure (MOF) caused by systemic inflammatory response syndrome [1]. Recent work suggests that detrimental modes of mechanical ventilation can contribute to MOF through the spread of inflammatory mediators [2, 3, 4, 5, 6]. Support for this hypothesis comes from both in vitro and clinical studies. In vitro, ventilation with approximately twice the normal tidal volume (i.e., 2 × 8 ml/kg) in isolated perfused mouse lungs elicits release of inflammatory...
mediators into the perfusate, i.e., the systemic circulation [3, 6]. Similarly, in isolated nonperfused rat lungs Tremblay and coworkers [5] demonstrated an increase in the release of inflammatory mediators after mechanical ventilation with large tidal volume (40 ml/kg bodyweight) without positive end-expiratory pressure (PEEP). In a clinical study Ranieri and colleagues [2] showed that mechanical ventilation with large tidal volume and low levels of PEEP leads to higher levels of cytokines both in the bronchoalveolar lavage (BAL) and serum than in patients ventilated with low tidal volumes and significantly higher levels of PEEP. In addition, in the ARDSnet [7, 8] ventilation study ventilation with reduced tidal volumes also resulted in lower mediator levels than conventional ventilation. In that study the PEEP levels used in the two groups were similar; these findings suggest that overstretching of alveolar units results in stimulation of the immune system which may be exacerbated by the lack of PEEP.

However, in contrast to the evidence cited above, in vivo ventilation alone in uninjured lungs does not appear to be a sufficient stimulus for mediator release. This was shown by studies both in rats [9] and in humans [10]. Altogether this suggests that ventilation-induced cytokine release in isolated organs, and more importantly in acute respiratory distress syndrome patients, may be explained by a two-hit model with ventilation being the second hit. One such hypothesis states that the degree of overstretching necessary to evoke cytokine release can only be achieved in isolated or inhomogeneously (pre)injured lungs. An alternative, although not mutually exclusive explanation for the spread of mediators as a result of ventilation, is loss of compartmentalization. The important concept of compartmentalization comprises the fact that the inflammatory response remains compartmentalized in the area of the body where it is produced, for example, in the alveolar space or in the systemic circulation [11, 12, 13, 14]. Tutor et al. [13] have shown in isolated lungs that compartmentalization of intra-alveolar tumor necrosis factor (TNF-α) may be lost after chemically induced lung injury.

The present study was undertaken to study the effect of ventilation on compartmentalization in vivo. Alveolar or systemic inflammation was therefore induced by either intratracheal or intraperitoneal injection of bacterial lipopolysaccharides (LPS) and used TNF-α as a marker to investigate the effect of various ventilation strategies on the compartmentalization of the inflammatory response.

### Materials and methods

This study was approved by the local Animal Committee of the Erasmus University Rotterdam. Care and handling of the animals were in accordance with the European Community guidelines. The studies were performed in male Sprague-Dawley rats (n = 85) with a bodyweight (BW) of 260 ± 40 g (IFFA Credo, The Netherlands). An overview of the various experimental groups is presented in Table 1.

#### Intratracheally treated animals

Two groups received LPS 5 ml/kg intratracheally (1 mg/ml Salmonella Abortus Equi S form, Melalon, Wusterhausen, Germany) and another two groups received the same amount of saline (5 ml/kg) intratracheally as previously described [15]. In short, animals treated intratracheally were anesthetized with 65% nitrous oxide/33% oxygen/2% isoflurane (Isoflurane; Pharmachemie, Haarlem, The Netherlands), and tracheotomized. A sterile metal cannula was inserted into the trachea; subsequently the operation area was infiltrated with 30 mg/kg lidocaine (xylocaine; Astra Pharmaceutical, Rijswijk, The Netherlands). LPS or saline was administered through the tracheal cannula over 5 min in spontaneously breathing animals; all animals recovered from anesthesia and breathed spontaneously for the ensuing 90 min.

#### Intraperitoneally treated animals

Animals receiving intraperitoneal administration of either LPS (15 ml/kg) or saline (15 ml/kg) were anesthetized using the same method as described above; however, no trachea cannula was inserted, and consequently there was no local infiltration with lidocaine. Animals recovered from anesthesia and breathed spontaneously during the ensuing 90 min.

#### Mechanical ventilation

All animals were anesthetized again with inhalation anesthesia (see previous description) and a sterile polyethylene catheter