Effect of ventilation strategy and surfactant on inflammation in experimental pneumonia

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ABSTRACT: This study explored, the inflammatory response during experimental pneumonia in surfactant-depleted animals as a function of ventilation strategies and surfactant treatment.

Following intratracheal instillation of Group B streptococci (GBS), surfactant-depleted piglets were treated with conventional (positive-end expiratory pressure (PEEP) of 5 cmH₂O, tidal volume 7 mL·kg⁻¹) or open lung ventilation. During the latter, collapsed alveoli were recruited by applying high peak inspiratory pressures for a short period of time, combined with high levels of PEEP and the smallest possible pressure amplitude. Subgroups in both ventilation arms also received exogenous surfactant. Conventionally ventilated healthy animals receiving GBS and surfactant-depleted animals receiving saline served as controls.

In contrast with both control groups, surfactant-depleted animals challenged with GBS and conventional ventilation showed high levels of interleukin (IL)-8, tumour necrosis factor (TNF)- α and myeloperoxidase in bronchoalveolar lavage fluid after 5 h of ventilation. Open lung ventilation attenuated this inflammatory response, but exogenous surfactant did not. Systemic dissemination of the inflammatory response was minimal, as indicated by low serum levels of IL-8 and TNF- α .

In conclusion, the current study indicates that the ventilation strategy, but not exogenous surfactant, is an important modulator of the inflammation during Group B streptococci pneumonia in mechanically ventilated surfactant-depleted animals.

KEYWORDS: Group B streptococcus, interleukin-8, lung protective ventilation, myeloperoxidase, surfactant, tumour necrosis factor- α

Animal studies have shown that mechanical ventilation (MV) can induce an inflammatory response in both the lung and the systemic compartment [1, 2]. This response is enhanced by applying high tidal volumes combined with low levels of positive end-expiratory pressure (PEEP), and attenuated by low tidal volumes and high levels of PEEP [1]. A recent study in adults confirmed these experimental findings [3].

However, the inflammatory response to MV is also strongly influenced by the presence or absence of pre-existing lung injury [4, 5]. Inducing surfactant-dysfunction or challenging the lung with endotoxin augments ventilation-induced inflammation [2, 5]. To date, no study has explored the inflammatory response to MV after exposing the lung to viable bacteria and/or surfactant depletion. These conditions are relevant as pneumonia is a common problem in ventilated intensive care patients [6–8], who often show signs of surfactant dysfunction [9, 10].

The aim of the present study was to determine to what extent different ventilation strategies and/

or natural modified surfactant modulate the inflammatory response upon exposing the lungs of newborn piglets to Group B streptococci (GBS) bacteria. Either, a conventional ventilation strategy (low tidal volumes and PEEP) or an open lung ventilation strategy was used, also aiming to recruit and stabilise the majority of previously collapsed alveoli (open lung concept (OLC)) [11]. To assess the impact of pre-existing lung injury, both healthy and surfactant-depleted animals were included.

The current authors hypothesised that conventional ventilation would enhance the inflammatory response in surfactant-depleted animals challenged with GBS and that both open lung ventilation and exogenous surfactant would attenuate this response.

To test this hypothesis the inflammatory response was assessed by measuring tumour necrosis factor (TNF)- α and interleukin (IL)-8 levels in lavage fluid and serum, and by assessing polymorphonuclear leukocyte (PMN) activation through myeloperoxidase (MPO) levels

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in lavage fluid. Data on bacterial growth and noninflammatory lung injury parameters were published previously [12].

METHODS

The study was approved by the institutional Animal Investigation Committee (Rotterdam, The Netherlands), and the experiments were performed according to the guidelines of the Helsinki convention.

Bacteria

Encapsulated GBS (Ia 90 LD) stored at -70°C were shifted to the mid-logarithmic growth phase as previously described [12]. The bacteria were then centrifugated, washed and resuspended in sterile saline at a concentration of $\sim 10^8$ colony forming units (cfu) per mL as determined by spectrophotometric measurement of the optical density at 595 nm. The exact number of viable cfu per mL in the GBS suspension was determined by serial dilution [13].

Animal preparation

Anesthesia was induced in 55 mixed-breed newborn piglets with a mean $\pm$ SD weight of 1.9 ± 0.3 kg with ketamine hydrochloride ($35 \text{ mg} \cdot \text{kg}^{-1}$, intramuscularly) and midazolam ($0.5 \text{ mg} \cdot \text{kg}^{-1}$, intramuscularly). The animals were tracheotomised and thereafter ventilated in the pressure controlled time-cycled mode (Servo 300; Siemens-Elma, Solna, Sweden). A neuromuscular block was induced with pancuronium bromide ($0.5 \text{ mg} \cdot \text{kg}^{-1}$, intravenously), followed by a continuous infusion of fentanyl ($20 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$), midazolam ($0.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and pancuronium bromide ($0.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$).

Using an aseptic technique, catheters were inserted into the external jugular vein for measurement of central venous pressure and infusion of fluids ($100 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{days}^{-1}$) and medication; and the carotid artery for monitoring of blood pressure and blood sampling.

Lavage procedure and surfactant treatment

In a subgroup of animals respiratory failure was induced by removing the endogenous surfactant through repeated saline lavage, as previously described [14].

Depending on the treatment group, animals received an endotracheal bolus of natural modified surfactant ($300 \text{ mg} \cdot \text{kg}^{-1}$; $50 \text{ mg} \cdot \text{mL}^{-1}$) or an equal volume ($6 \text{ mL} \cdot \text{kg}^{-1}$) of air. The surfactant (HL 10; Leo Pharmaceutical Products, Ballerup, Denmark; Halas Pharma GmbH, Oldenburg, Germany) used contained 98% lipids (mainly phospholipids) and 1–2% hydrophobic proteins SP-B and SP-C.

GBS instillation

Thirty minutes after the administration of either surfactant or air bolus, the animals received two aliquots of $5 \text{ mL} \cdot \text{kg}^{-1}$ of the GBS suspension, slowly injected through a catheter placed at the end of the endotracheal tube in the right and left lateral position to ensure equal distribution. Following this procedure the animals were returned to the supine position for the remainder of the experiments.

Ventilation strategies

All animals received positive pressure ventilation, but depending on the treatment group they were subjected to one of two

ventilation strategies. The ventilation time was 5 h following GBS instillation and during this time fractional inspired oxygen (F_{I,O_2}) was kept at 1.0.

Conventional positive pressure ventilation

During conventional positive pressure ventilation (PPVCON) animals were ventilated in the pressure controlled mode. The peak inspiratory pressure (PIP) was set at a level that resulted in an expiratory tidal volume of $\sim 7 \text{ mL} \cdot \text{kg}^{-1}$, measured at the Y-piece (CO₂SMO Plus; Novamatrix Systems, Wallingford, CT, USA). The level of PEEP was maximised at $5 \text{ cmH}_2\text{O}$ and the ventilatory rate could be adjusted between $30\text{--}60 \text{ breaths} \cdot \text{min}^{-1}$ in order to prevent hypercapnia (carbon dioxide arterial tension $>55 \text{ mmHg}$).

Open lung concept positive pressure ventilation

As previously described, the main objectives of the open lung concept positive pressure ventilation (PPVOLC) strategy is to recruit atelectatic lung regions using high levels of PIP for a short period of time, and to prevent repeated alveolar collapse by applying sufficient levels of PEEP [14]. Changes in intrapulmonary shunt and subsequent changes in oxygenation were used to assess alveolar collapse. For this reason, a sensor for continuous blood gas monitoring (Paratrend; Diametrics Medical Ltd, High Wycombe, UK) was inserted through a femoral artery catheter. Based on arterial oxygen tension (P_{a,O_2}) levels in the healthy piglets ventilated with a F_{I,O_2} concentration of 1.0, optimal alveolar recruitment was defined when $P_{a,O_2} \geq 59.9 \text{ kPa}$. Starting at a level of $5 \text{ cmH}_2\text{O}$, the PEEP was stepwise increased while maintaining a fixed pressure amplitude (PIP minus PEEP) of $10\text{--}12 \text{ cmH}_2\text{O}$. Once the P_{a,O_2} reached 59.9 kPa (open lung), the PEEP was stepwise reduced until the P_{a,O_2} deteriorated, indicating progressive alveolar collapse (closing pressure). The lung was once again recruited and the PEEP was set at $2 \text{ cmH}_2\text{O}$ above the closing pressure, thus applying the lowest possible PEEP to maintain an open lung. The pressure amplitude was minimised as much as possible in order to prevent alveolar over-distension, and hypercapnia was prevented by using supranormal ventilatory rates ($120 \text{ breaths} \cdot \text{min}^{-1}$).

Experimental groups

After the instrumentation period the animals were randomly allocated to one of the following groups, each consisting of 10 animals, unless stated differently.

Healthy

These healthy animals received an intratracheal bolus of air, the GBS solution and were subsequently ventilated according to the PPVCON strategy.

Lavaged

Animals in this group were subjected to lung lavage and subsequently received an intratracheal bolus of air, the GBS solution and PPVCON.

Surfactant

Animals in this group received an intratracheal bolus of exogenous surfactant after the lavage procedure, followed by the GBS solution and PPVCON.

OLC

Animals in this group were lavaged, received an intratracheal bolus of air, the GBS solution and were ventilated according to the PPVOLC strategy.

Surfactant-OLC

The lavaged animals in this group received exogenous surfactant, the GBS solution and were also ventilated according to the PPVOLC strategy.

Saline

The five animals in this control group received a bolus of air following lung lavage, but instead of the GBS solution, 10 mL·kg⁻¹ of sterile saline was instilled in a similar fashion. Ventilation was according to the PPVCON strategy.

Inflammatory analysis

At the end of the experiments, 3 mL of blood was drawn for analysis of inflammatory parameters. Bronchoalveolar lavage (BAL) was then performed on the right lung with 40 mL·kg⁻¹ saline solution supplemented with 1.2 mM CaCl₂. The percentage of lung lavage fluid recovered was calculated. Blood and lavage samples were spun for 10 min at 1,500 × g to remove cell material, and thereafter stored at -80°C.

IL-8, TNF-α and MPO were measured in ELISA; IL-8 and TNF-α in assays using antibodies directed against porcine IL-8 and TNF-α (pIL-8 and pTNF-α, respectively), and MPO in an assay using antibodies raised against human MPO.

For pTNF-α, Nunc maxisorp ELISA plates were coated overnight at 4°C with a polyclonal rabbit anti-mouse immunoglobulin G (Z0412; DAKO, Glostrup, Denmark; 0.42 µg·mL⁻¹; 100 µL per well). Plates were washed four times with 0.02% (volume (v)/v) Tween-20 in PBS (washing buffer), after which remaining binding sites were blocked using 1% (weight (w)/v) bovine serum albumin (BSA; Sigma, St. Louis, MO, USA), and 5% (w/v) sucrose in PBS (200 µL per well) under constant agitation (500 rpm) for 1 h at room temperature. Plates were washed four times with washing buffer and incubated with MAB 6902 (R&D systems, Uithoorn, The Netherlands; 1 µg·mL⁻¹, 100 µL per well) at 500 rpm for 1 h at room temperature.

After four washes, samples (200 µL per well) were diluted at least 1:1 with 1% BSA in 20 mM Trizma base. As a standard 150 mM NaCl, pH 7.3 or recombinant pTNF-α (R&D) were added to the wells and left overnight at room temperature and 500 rpm. The plates were washed four times, and the detecting biotinylated antibody (BAF 690; R&D; 250 ng·mL⁻¹, 200 µL per well) was added and left for 2 h at room temperature and 500 rpm in the dark. At least 1 h prior to use of BAF 690, the antibody was diluted in 1% BSA with 2% (v/v) normal goat serum in PBS. After incubation, plates were washed four times, incubated for 30 min at room temperature and 500 rpm with streptavidin-poly-HRP (Sanquin, Amsterdam, The Netherlands; 1/10,000 dilution in 2% (v/v) milk in PBS, 200 µL per well). After four washes, plates were developed with tetramethylbenzidine and read in a microtitreplate reader. For pIL-8 the same protocol as described above was used. MAB 5351 and BAF 535 (both R&D) were used as catching and detecting antibodies, respectively. Samples were diluted in 1%

BSA in PBS, and no normal goat serum was used in the second antibody step. The MPO ELISA has been described elsewhere [15].

Although the polyclonal antibodies used in this assay were raised against human MPO, a recent study showed a high degree of homology between human and porcine MPO (pMPO) [16]. Consistent with this observation, serial dilutions of porcine material paralleled the standard curve with human MPO, indicating that this assay could be used to detect pMPO.

The lower limits of detections for pIL-8, pTNF-α and pMPO were 0.08, 0.4 and 2 ng·mL⁻¹, respectively. For all assays, recovery studies were performed in which exogenous protein added to a sample was recovered near 100%.

Statistical analysis

Data on IL-8, TNF-α and MPO were presented as scatter plots and analysed with a Kruskal Wallis test, followed by Dunn's multiple comparison test. For statistical purposes, levels below the detection limit were set at zero. The Spearman rank coefficient was used to analyse correlations between these variables. A p-value of ≤0.05 was considered statistically significant.

RESULTS

Animals

All animals that were included completed the study. There were no intergroup differences in age and weight. The recovery of BAL fluid was 64±6% of the volume instilled and this was comparable in all groups.

Inflammatory parameters

As shown in figures 1, 2 and 3, pulmonary inflammation measured by IL-8, MPO and TNF-α in lavage fluid was

FIGURE 1. Scatter plot of individual interleukin (IL-8) levels in the lavage fluid obtained at the end of the ventilation period. Healthy: Group B streptococci (GBS)+conventional positive pressure ventilation (PPVCON; ●); Lavaged: lavaged+GBS+PPVCON (○); Surfactant: lavaged+GBS+surfactant+PPVCON (■); Open lung concept (OLC): lavaged+GBS+open lung concept positive pressure ventilation (PPVOLC; □); Surfactant-OLC: lavaged+GBS+surfactant+PPVOLC (◆); Saline: lavaged+saline+PPVCON (◇). DL: lower detection limit. —: median. **: p<0.01 versus healthy and p<0.05 versus saline. ***: p<0.001 versus healthy and p<0.01 versus saline.

FIGURE 2. Scatter plot of individual myeloperoxidase levels in the lavage fluid obtained at the end of the ventilation period. Healthy: Group B streptococci (GBS)+conventional positive pressure ventilation (PPVCON; ●); Lavaged: lavaged+GBS+PPVCON (○); Surfactant: lavaged+GBS+surfactant+PPVCON (■); Open lung concept (OLC): lavaged+GBS+open lung concept positive pressure ventilation (PPVOLC; □); Surfactant-OLC: lavaged+GBS+surfactant+PPVOLC (◆); Saline: lavaged+saline+PPVCON (◇). DL: lower detection limit. —: median. *: $p < 0.05$ versus saline. ***: $p < 0.001$ versus saline and $p < 0.05$ versus OLC, surfactant-OLC.

minimal in conventionally ventilated surfactant-depleted animals not challenged with GBS bacteria (saline). This observation was also true for healthy animals who received GBS (healthy), except that MPO levels were somewhat higher compared with the saline group, indicating PMN activation (fig. 2). However, combining surfactant-depletion and GBS instillation during PPVCON (lavaged), resulted in a significant

FIGURE 3. Scatter plot of individual tumour necrosis- α levels in the lavage fluid obtained at the end of the ventilation period. Healthy: Group B streptococci (GBS)+conventional positive pressure ventilation (PPVCON; ●); Lavaged: lavaged+GBS+PPVCON (○); Surfactant: lavaged+GBS+surfactant+PPVCON (■); Open lung concept (OLC): lavaged+GBS+open lung concept positive pressure ventilation (PPVOLC; □); Surfactant-OLC: lavaged+GBS+surfactant+PPVOLC (◆); Saline: lavaged+saline+PPVCON (◇). DL: lower detection limit. —: median. *: $p < 0.05$ versus surfactant-OLC. **: $p < 0.01$ versus healthy. ***: $p < 0.001$ versus healthy and $p < 0.05$ versus saline, OLC.

increase of IL-8, MPO and TNF- α levels in the lavage fluid. Applying an open lung ventilation strategy under these conditions (OLC) prevented this inflammatory response almost completely, whereas treatment with exogenous surfactant (surfactant) did not. Combining surfactant treatment and open lung ventilation (surfactant-OLC) did not further modulate IL-8 and MPO levels in the lavage fluid. However, TNF- α levels significantly increased in response to this combined treatment (fig. 3).

Exploring possible correlations between the inflammatory changes in the lavage fluid revealed a significant correlation between the IL-8 and MPO levels using all data (fig. 4).

Serum levels of IL-8 were detectable in a minority of animals in the healthy ($n=5$), lavaged ($n=4$), surfactant ($n=5$), OLC ($n=2$) and surfactant-OLC ($n=1$) groups. However, the median levels for each group were low (<0.5 ng·mL $^{-1}$) and revealed no significant differences between the groups (data not shown). Serum TNF- α was detectable only in one animal in the surfactant, OLC and surfactant-OLC group. The levels were just above the detection limit (data not shown).

DISCUSSION

This is the first study exploring the effect of viable bacteria and surfactant depletion on local and systemic inflammation in response to various MV strategies and exogenous surfactant. The study has shown that PPVCON induced a marked inflammatory response with increased BAL fluid levels of IL-8, TNF- α and MPO in surfactant-depleted animals with GBS pneumonia. This pro-inflammatory response was prevented by using a lung protective ventilation strategy, *i.e.* open lung ventilation, but not by administration of exogenous surfactant.

It was interesting to observe that conventional ventilation of surfactant-depleted animals in the saline control group did not stimulate pulmonary inflammation, resulting in low levels of IL-8, TNF- α and MPO in lavage fluid. This is probably best explained by the fact that the settings applied during PPVCON in the present study (low tidal volumes and PEEP), are nowadays considered noninjurious [17, 18]. However, when

FIGURE 4. Correlation between individual levels of interleukin (IL)-8 and myeloperoxidase in lavage fluids obtained at the end of the ventilation period ($r_s=0.58$; $p < 0.0001$).

inducing a “second hit” with the instillation of GBS in the surfactant-depleted lung, these same settings during PPVCON resulted in a profound pro-inflammatory response.

It is possible that this augmented inflammatory response is mediated by direct stimulation of alveolar macrophages by GBS bacteria, as shown by previous *in vitro* experiments [19]. However, the low levels of TNF- α and IL-8 in healthy animals challenged with GBS, suggests that this pathway was probably of lesser importance in the present experimental setting. Instead, GBS bacteria activate resident PMN, as indicated by increased MPO levels, which may enhance subsequent inflammatory responses.

A second explanation for this augmented response could be an increased susceptibility to ventilator-induced lung injury during conventional ventilation after challenging the surfactant-depleted lung with GBS bacteria. Both *in vitro* and *in vivo* studies have shown that endotoxin enhances chemokine release from pulmonary immune cells in response to cyclic stretch or MV, even when low tidal volumes were used comparable with the present study (7 mL·kg⁻¹) [5, 20]. In line with this latter reasoning is the current authors observation that open lung ventilation prevented the enhanced inflammatory response following surfactant depletion and GBS instillation. The most important difference with the low tidal volume PPVCON group, is the application of a recruitment manoeuvre and higher levels of PEEP to open up and stabilise previously collapsed alveoli. This approach not only minimises alveolar stretch, but also prevents repetitive opening and collapse (atelectrauma), which is also considered an important mechanism in ventilator-induced lung injury [21].

In addition to different ventilation strategies, the present study also explored the effect of exogenous surfactant on the inflammatory response during experimental pneumonia. It was found that exogenous surfactant did not attenuate this response. This contrasts with previous *in vivo* experiments showing that exogenous surfactant reduced ventilator-induced lung injury [22, 23], and *in vitro* studies indicating that surfactant suppressed the release of mediators, such as TNF- α and IL-8 from stimulated alveolar macrophages [24]. The increased TNF- α production after administering exogenous surfactant to OLC ventilation might even suggest that surfactant is able to stimulate cytokine production during pneumonia. Similar findings have also been reported for IL-8, IL-6 and TNF- α in other models of lung injury [22, 25]. The current authors can only speculate on the reasons as to why exogenous surfactant did not attenuate the inflammatory response in this pneumonia model. First, studies that showed reduced cytokine secretion from stimulated pulmonary cells in the presence of surfactant were carried out *in vitro*, while the present study was carried out *in vivo*. As previously shown, these different environmental conditions can influence cytokine levels in the ventilated lung [4]. Secondly, in contrast to the *in vitro* studies, the cells in the lungs in the present study were also subjected to cyclic stretch due to MV. As mentioned previously, stretching of pulmonary cells increases the release of cytokines [20]. It has even been suggested that exogenous surfactant augments this stretch-induced cytokine release as it improves the mechanical properties of the lung [25].

It is conceivable that this stretch-induced cytokine release overwhelmed the mitigating effect of exogenous surfactant on cytokine production as shown *in vitro*.

The correlation between IL-8 and MPO indicates that IL-8 at large is responsible for the recruitment of PMN, although other chemotactic substances in the pulmonary alveoli are also able to attract and activate PMN [26]. The present authors did not observe a clear correlation between IL-8 and TNF- α levels in the lavage fluid (data not shown), indicating that TNF- α and IL-8 production are regulated independently during the early phase of pneumonia in these short-term experiments.

Previous animal studies have shown that MV of a surfactant-depleted lung can result in decompartmentalisation of cytokines produced in the lung [2, 27]. However, in the present study low serum levels of TNF- α and IL-8 were found in all groups, despite the high cytokine levels in lavage fluid. This discrepancy is probably best explained by the fact that these previous experiments used injurious ventilation settings, *i.e.* high tidal volumes and zero PEEP [2, 27]. This is in contrast with the low tidal volume and PEEP applied during PPVCON in the present study. Although this strategy was not able to attenuate TNF- α and IL-8 production in the lung, it was able to prevent release of these cytokines into the systemic circulation. An alternative explanation for the low serum cytokine levels could be the short duration of the present experiment, which may have been too short for decompartmentalisation to actually take place.

Studies in ventilated intensive care patients have also reported increased levels of TNF- α and IL-8 in the BAL fluid during pneumonia [28–30]. Some have even suggested that high IL-8 levels are associated with increased mortality in patients with pneumonia [28]. The results from the present study do not allow such conclusions, as the authors studied only the early inflammatory changes in GBS pneumonia. In addition, extrapolation of animal data to humans should be done with caution. Nevertheless, the current study shows that application of low tidal volumes during MV is not sufficient to attenuate the early inflammatory response during pneumonia. Recruiting the lung and stabilising opened alveoli might prove essential in damping this early response. Future studies, covering a larger time scale, need to extend and confirm these findings.

In conclusion, the present study shows that inflammatory changes during pneumonia in surfactant-depleted ventilated piglets are strongly influenced by the ventilation strategy. Conventional ventilation induces a marked inflammatory response (interleukin-8, tumour necrosis factor- α and myeloperoxidase) in the lung, but not in the systemic compartment. Open lung ventilation, but not exogenous surfactant, attenuates this response.

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REFERENCES

- 1 Tremblay L, Valenza F, Ribeiro SP, Li J, Slutsky AS. Injurious ventilatory strategies increase cytokines and c-fos mRNA expression in an isolated rat lung model. *J Clin Invest* 1997; 99: 944–952.
- 2 Chiumello D, Pristine G, Slutsky AS. Mechanical ventilation affects local and systemic cytokines in an animal model of acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1999; 160: 109–116.
- 3 Ranieri VM, Suter PM, Tortorella C, et al. Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: a randomized controlled trial. *JAMA* 1999; 282: 54–61.
- 4 Ricard JD, Dreyfuss D, Saumon G. Production of inflammatory cytokines in ventilator-induced lung injury: a reappraisal. *Am J Respir Crit Care Med* 2001; 163: 1176–1180.
- 5 Whitehead TC, Zhang H, Mullen B, Slutsky AS. Effect of mechanical ventilation on cytokine response to intratracheal lipopolysaccharide. *Anesthesiology* 2004; 101: 52–58.
- 6 Vincent JL, Bihari DJ, Suter PM, et al. The prevalence of nosocomial infection in intensive care units in Europe. Results of the European Prevalence of Infection in Intensive Care (EPIC) Study. EPIC International Advisory Committee. *JAMA* 1995; 274: 639–644.
- 7 Grohskopf LA, Sinkowitz-Cochran RL, Garrett DO, et al. A national point-prevalence survey of pediatric intensive care unit-acquired infections in the United States. *J Pediatr* 2002; 140: 432–438.
- 8 Nagata E, Brito AS, Matsuo T. Nosocomial infections in a neonatal intensive care unit: incidence and risk factors. *Am J Infect Control* 2002; 30: 26–31.
- 9 Gunther A, Siebert C, Schmidt R, et al. Surfactant alterations in severe pneumonia, acute respiratory distress syndrome, and cardiogenic lung edema. *Am J Respir Crit Care Med* 1996; 153: 176–184.
- 10 Griesse M, Westerburg B, Potz C, Dietrich P. Respiratory support, surface activity and protein content during nosocomial infection in preterm neonates. *Biol Neonate* 1996; 70: 271–279.
- 11 van Kaam AH, De Jaegere A, Haitsma JJ, Van Aalderen WM, Kok JH, Lachmann B. Positive pressure ventilation with the open lung concept optimizes gas exchange and reduces ventilator-induced lung injury in newborn piglets. *Pediatr Res* 2003; 53: 245–253.
- 12 van Kaam AH, Lachmann RA, Herting E, et al. Reducing atelectasis attenuates bacterial growth and translocation in experimental pneumonia. *Am J Respir Crit Care Med* 2004; 169: 1046–1053.
- 13 Herting E, Jarstrand C, Rasool O, Curstedt T, Sun B, Robertson B. Experimental neonatal group B streptococcal pneumonia: effect of a modified porcine surfactant on bacterial proliferation in ventilated near-term rabbits. *Pediatr Res* 1994; 36: 784–791.
- 14 van Kaam AH, Dik WA, Haitsma JJ, et al. Application of the open-lung concept during positive-pressure ventilation reduces pulmonary inflammation in newborn piglets. *Biol Neonate* 2003; 83: 273–280.
- 15 Bresser P, Out TA, van Alphen L, Jansen HM, Lutter R. Airway inflammation in nonobstructive and obstructive chronic bronchitis with chronic haemophilus influenzae airway infection. Comparison with noninfected patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000; 162: 947–952.
- 16 Marklund L, Kaul S, Tuggle CK. Rapid communication: Mapping of the myeloperoxidase (MPO) gene to pig chromosome 12. *J Anim Sci* 2000; 78: 3189–3190.
- 17 Courtney SE, Durand DJ, Asselin JM, Hudak ML, Aschner JL, Shoemaker CT. High-frequency oscillatory ventilation versus conventional mechanical ventilation for very-low-birth-weight infants. *N Engl J Med* 2002; 347: 643–652.
- 18 The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med* 2000; 342: 1301–1308.
- 19 Kwak DJ, Augustine NH, Borges WG, Joyner JL, Green WF, Hill HR. Intracellular and extracellular cytokine production by human mixed mononuclear cells in response to group B streptococci. *Infect Immun* 2000; 68: 320–327.
- 20 Pugin J, Dunn I, Jolliet P, et al. Activation of human macrophages by mechanical ventilation *in vitro*. *Am J Physiol* 1998; 275: Suppl. 6 Pt 1, L1040–L1050.
- 21 Dreyfuss D, Saumon G. Ventilator-induced lung injury: lessons from experimental studies. *Am J Respir Crit Care Med* 1998; 157: 294–323.
- 22 van Kaam AH, Haitsma JJ, Dik WA, et al. Response to exogenous surfactant is different during open lung and conventional ventilation. *Crit Care Med* 2004; 32: 774–780.
- 23 Verbrugge SJ, Vazquez de Anda GF, Gommers D, et al. Exogenous surfactant preserves lung function and reduces alveolar Evans blue dye influx in a rat model of ventilation-induced lung injury. *Anesthesiology* 1998; 89: 467–474.
- 24 Thomassen MJ, Antal JM, Connors MJ, Meeker DP, Wiedemann HP. Characterization of exosurf (surfactant)-mediated suppression of stimulated human alveolar macrophage cytokine responses. *Am J Respir Cell Mol Biol* 1994; 10: 399–404.
- 25 Stamme C, Brasch F, von Bethmann A, Uhlig S. Effect of surfactant on ventilation-induced mediator release in isolated perfused mouse lungs. *Pulm Pharmacol Ther* 2002; 15: 455–461.
- 26 Sherman MP, Ganz T. Host defense in pulmonary alveoli. *Annu Rev Physiol* 1992; 54: 331–350.
- 27 Haitsma JJ, Uhlig S, Goggel R, Verbrugge SJ, Lachmann U, Lachmann B. Ventilator-induced lung injury leads to loss of alveolar and systemic compartmentalization of tumor necrosis factor- α . *Intensive Care Med* 2000; 26: 1515–1522.
- 28 Chollet-Martin S, Jourdain B, Gibert C, Elbim C, Chastre J, Gougerot-Pocidalo MA. Interactions between neutrophils and cytokines in blood and alveolar spaces during ARDS. *Am J Respir Crit Care Med* 1996; 154: 594–601.
- 29 Buck C, Gallati H, Pohlandt F, Bartmann P. Increased levels of tumor necrosis factor α (TNF- α) and interleukin 1 β (IL-1 β) in tracheal aspirates of newborns with pneumonia. *Infection* 1994; 22: 238–241.
- 30 Millo JL, Schultz MJ, Williams C, et al. Compartmentalisation of cytokines and cytokine inhibitors in ventilator-associated pneumonia. *Intensive Care Med* 2004; 30: 68–74.