Intensive Care Med (2002) 28:486–492
DOI 10.1007/s00134-002-1216-7

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Mechanical ventilation alters the immune response in children without lung pathology

Received: 3 March 2001
Accepted: 28 December 2001
Published online: 9 February 2002

F. Plötz was funded by the Wijnand M. Pon Foundation and the Catharijne Foundation, University Medical Center Utrecht

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Abstract *Objective:* This study was undertaken to examine the hypothesis that mechanical ventilation in association with anesthesia would alter the cytokine profile in infants without preexisting lung pathology. *Design and setting:* Prospective observational study in pediatric intensive care unit in a university hospital. *Patients:* Twelve infants who were subjected to an uncomplicated diagnostic cardiac catheterization procedure were studied. All subjects were ventilated with a volume control mode, 0.3 FIO_2, 4 cmH_2O PEEP, and 10 ml/kg tidal volume. Volatile (servoflurane) anesthetics were given. *Measurements and results:* Tracheal aspirates and blood samples were obtained before and after 2 h of mechanical ventilation. In tracheal aspirates and in supernatants of stimulated whole-blood cultures cytokine concentrations were measured. In the tracheal aspirates the immune balance was characterized by a proinflammatory response pattern, with a significant increase in TNF-α and IL-6 concentrations; concentrations of anti-inflammatory mediators remained very low. The functional capacity of peripheral blood leukocytes to produce INF-γ, TNF-α, and IL-6 in vitro was significantly decreased. This was accompanied by a significant decrease in the killing activity of natural killer cells. *Conclusions:* Two hours of servoflurane and mechanical ventilation using a tidal volume of 10 ml/kg is associated with remarkable changes in the immune response in infants without preexisting lung pathology undergoing cardiac catheterization. In the lungs the immune balance favors a proinflammatory response pattern without detectable concentrations of anti-inflammatory mediators. The Th1 immune response by peripheral blood leukocytes was decreased. The observed change in Th1/Th2 balance in favor of Th2 cytokine activity may be a systemic adaptation to the proinflammatory milieu in the lung.

Keywords Mechanical ventilation · Cardiac catheterization · Interleukins · Tumor necrosis factor α · Interferon γ · Natural killer cells · Volatile anesthetics

Introduction

It has become clear that alterations in the immune balance may prevent an appropriate and effective response to various stimuli [1, 2]. $CD4^+$ T-cells can be divided functionally into Th1 and Th2 cells based on their cytokine profiles [3]. Th1 cells secrete interferon (IFN) γ while Th2 cells secrete interleukin (IL) 4, IL-5, IL-10, and IL-13. Macrophages secrete proinflammatory and anti-inflammatory cytokines such as IL-1β, tumor necro-

sis factor (TNF) α, IL-12, and IL-10. For example, an alteration in the Th1/Th2 balance, resulting in a Th2 dominance, is thought to contribute to enhanced pulmonary disease in respiratory syncytial virus bronchiolitis [4]. On the other hand, new evidence indicates that a disturbance of the balance between proinflammatory mediators and anti-inflammatory mediators may initiate or amplify the inflammatory response in patients with the acute respiratory distress syndrome (ARDS) [1, 5]. For example, the ratio of IL-1β to IL-1 receptor antagonist is markedly elevated in patients with ARDS, favoring the unopposed proinflammatory activity of IL-1β. The observation that low intrapulmonary concentrations of IL-10 and IL-1 receptor antagonist at the onset of ARDS are associated with a poor outcome suggests that a lack of inhibitory cytokines is correlated with a poor prognosis.

It has also been suggested that mechanical ventilation produces alterations in the immune balance [6]. Experimental studies have demonstrated that mechanical ventilation results in an inflammatory reaction in the lungs and that the degree of inflammation depends on the ventilatory strategy and mode [7, 8, 9, 10, 11]. This inflammatory reaction may not be limited to the lungs but may initiate or propagate multiple system organ failure [12, 13, 14]. A possible explanation for the spillover of inflammatory mediators as a result of mechanical ventilation is loss of compartmentalization [15]. The important concept of compartmentalization refers to the fact that the inflammatory response remains compartmentalized in the area of the body were it is produced [16, 17]. Haitsma et al. [18] have shown in rats that injurious ventilatory strategies, although not conclusive, disturb the compartmentalization of the early cytokine response in both the lung and the systemic circulation [15].

Infants who undergo cardiac catheterization may have multiple risk factors that may affect the inflammatory milieu in their lungs, including mechanical ventilation, exposure to anesthetic agents, and the stress of the procedure. The present study was designed to examine the hypothesis that mechanical ventilation in association with anesthesia would alter the cytokine profile in the lungs, and/or systemic circulation, of patients without preexisting lung pathology.

Material and methods

Study population

The study included 12 children (median age 3.5 years, range 1–11) who were undergoing a diagnostic cardiac catheterization procedure. The children had a history of a congenital heart disease, some of whom had been (partially) corrected: atrial-ventricular septal defect, transposition of the great arteries [2], aortic valve insufficiency, ventricular septal defect [2], tetralogy of Fallot, coarctation of aorta, tricuspid atresia, pulmonary atresia [2], double outlet right ventricle. Patients with a history of allergic or respiratory diseases, known chromosomal or immunological disorders, and patients recently hospitalized or mechanically ventilated were excluded. All subjects were intubated and ventilated with a volume control mode and a fractional inspiratory oxygen of 0.3, a maximum peak inspiratory pressure of 19.1±2.0 cm H_2O, a mean positive end-expiratory pressure (PEEP) of 3.8±1.0 cm H_2O, and a mean tidal volume of 9.95±0.95 ml/kg (measured body weight). The end-tidal CO_2 was maintained between 35–45 mmHg. If PEEP, inspiratory oxygen concentration, or tidal volume needed to be adjusted to maintain an adequate oxygenation or to maintain normocapnia, the patient was excluded from the study. Heart rate and blood pressure of the individual patients remained constant during the procedure. All patients received servoflurane (3.75%) anesthetic during the procedure. The study was approved by the Medical Ethics Committee, and parents gave informed consent.

Collection of materials

Tracheal aspirates and blood samples were obtained immediately after intubation, before the start of mechanical ventilation, and after 2 h of mechanical ventilation. Tracheal aspirates were obtained as previously described [19]. The suction catheter was rinsed with 0.5 ml sterile normal saline and added to the suction trap. The aspirate was placed immediately on ice. Thereafter 10% dithiothreitol (10%; 100 µl per 1 ml aspirate) was added, and the samples were centrifuged at 1500 rpm for 5 min. Supernatants were stored at –80°C until analysis. Blood samples were drawn from a venous catheter.

Cell cultures

Heparinized blood was diluted 1:10 in RPMI-1640 medium (Roswell Park Memorial Institute Life Technologies, Grand Island, N.Y., USA), and whole-blood cultures were set up. The whole-blood culture stimulated with lipopolysaccharide (LPS) is a suitable ex vivo method to study monocyte cytokine production under conditions in which many of the physiologically relevant cellular interactions remain intact [20, 21]. To induce lymphocyte cytokine production (IL-4, IFN-γ) anti-CD2,1 and anti-CD2,2 (1:12000) plus anti-CD28 (1:3000) monoclonal antibodies (CLB, Amsterdam, The Netherlands) were added, and cultures were incubated for 72 h at 37°C in 5% CO_2 in air. All cultures were performed in quadruplicate. To induce the production of monocyte IL-6, IL-8, TNF-α, LPS (Difco Laboratories, Detroit, Mich., USA) (1 ng/ml) was added to the diluted blood samples and cultures were incubated for 24 h at 37°C in 5% CO_2 in air. To induce monocyte IL-10 production LPS (1 ng/ml) was added, and cultures were incubated for 48 h at 37°C in 5% CO_2 in air. To induce monocyte IL-12 production LPS (100 ng/ml) and IFN-γ (20 ng/ml) were added, and cultures were incubated for 24 h at 37°C in 5% CO_2. Addition of IFN-γ results in a more optimal IL-12 response in the presence of LPS.

Cytokine assays

TNF-α, IL-4, IL-6, IL-8, IL-10, IL-12, and IFN-γ were measured via enzyme-linked immunosorbent assay (CLB). The detection limit was 4–6 pg/ml for TNF-α, 0.6 pg/ml for IL-4, 1 pg/ml for IL-6, 4–8 pg/ml for IL-8, 3–5 pg/ml for IL-10, 3 pg/ml for IL-12, and 4–6 pg/ml for IFN-γ. When cytokines were not detectable, the minimum detectable level was used in the calculations.

Cellular composition of blood

The composition of peripheral leukocytes was determined by analyzing the forward-sideward scatter. For lymphocyte subset analysis, whole blood was incubated with conjugated monoclonal antibodies under saturating conditions specific for CD3, CD4, CD8,

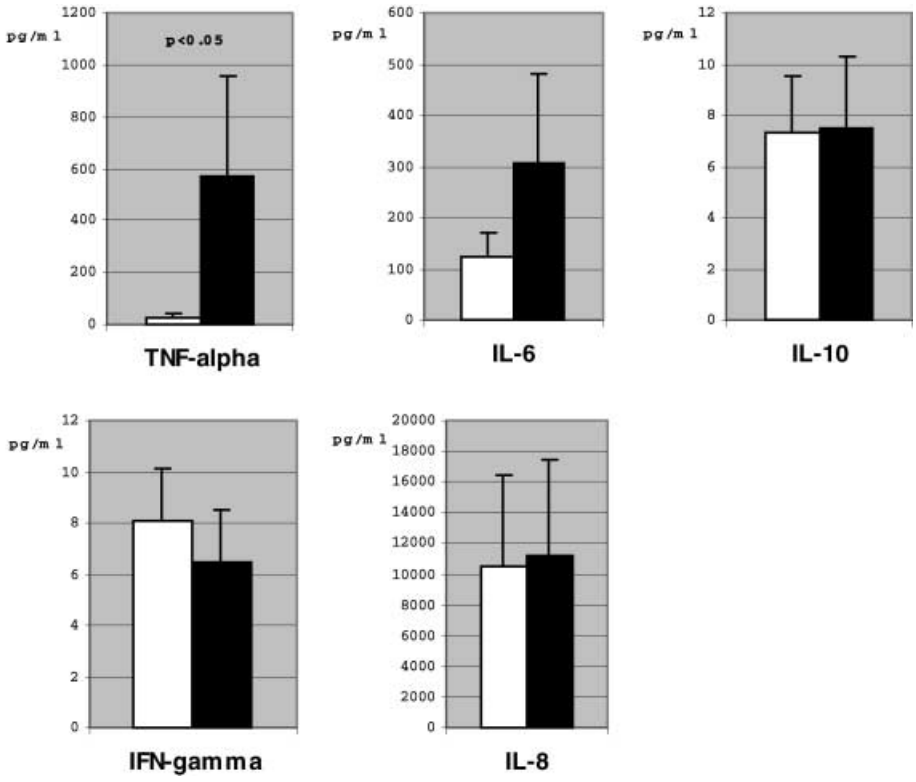

Fig. 1 Cytokine concentrations (mean ±SEM) in tracheal aspirates before (*open bars*) and after 2 h (*solid bars*) of mechanical ventilation

and CD16/56 (Simultest, Becton and Dickinson, Mountain View, Calif., USA). Subsequently, red blood cells were lysed and samples were analyzed with a flow cytometer (FACS-Star+, Becton and Dickinson). The difference between negative and positive fluorescence was determined by measuring unstained cells and cells stained with an irrelevant isotype control body.

Natural killer cell activity

Natural killer cell (NK) cell activity was analyzed by determining the capacity of peripheral blood cells to kill ^{51}Cr-labeled K562 target cells as described previously [22].

Plasma cortisol

Cortisol was measured by a chemiluminescence immunoassay performed on the fully automated ADVIA Centaur immunoanalyzer (Bayer, Leverkusen, Germany).

Statistical analysis

All values were expressed as mean ±SD and were analyzed by the nonparametric Wilcoxon signed-rank test. Differences were considered significant at the level of $p<0.05$.

Results

Tracheal aspirates

The concentrations of TNF-α in the supernatant of the tracheal aspirates increased significantly 2 h after mechanical ventilation ($p=0.01$; Fig. 1). There was a trend towards an increase in IL-6 levels ($p=0.05$; Fig. 1). IL-8 concentrations showed high interindividual variation both before and after mechanical ventilation. The concentrations of the anti-inflammatory cytokines IL-10 and IFN-γ remained unchanged just above the detection level (Fig. 1).

Blood samples

The capacity of lymphocytes to produce cytokines was determined in whole-blood cultures stimulated with anti-CD2/CD28 [20, 21]. After 2 h of mechanical ventilation a significant decrease in IFN-γ production was observed in the cultured supernatants ($p=0.01$), but no significant changes in IL-4 concentrations were observed (Fig. 2). The capacity of monocytes to produce cytokines was determined in whole-blood cultures stimulated with LPS. After 2 h of mechanical ventilation there was a decrease in the production of proinflammatory cytokines IL-6 ($p<0.05$) and TNF-α by peripheral blood monocytes ($p<0.05$; Fig. 2). IL-8 concentrations showed high interindividual variation before and after mechanical ventilation. The amount of IL-10 and IL-12 produced by monocytes was unaltered in all patients as a result of 2 h of mechanical ventilation (Fig. 2).

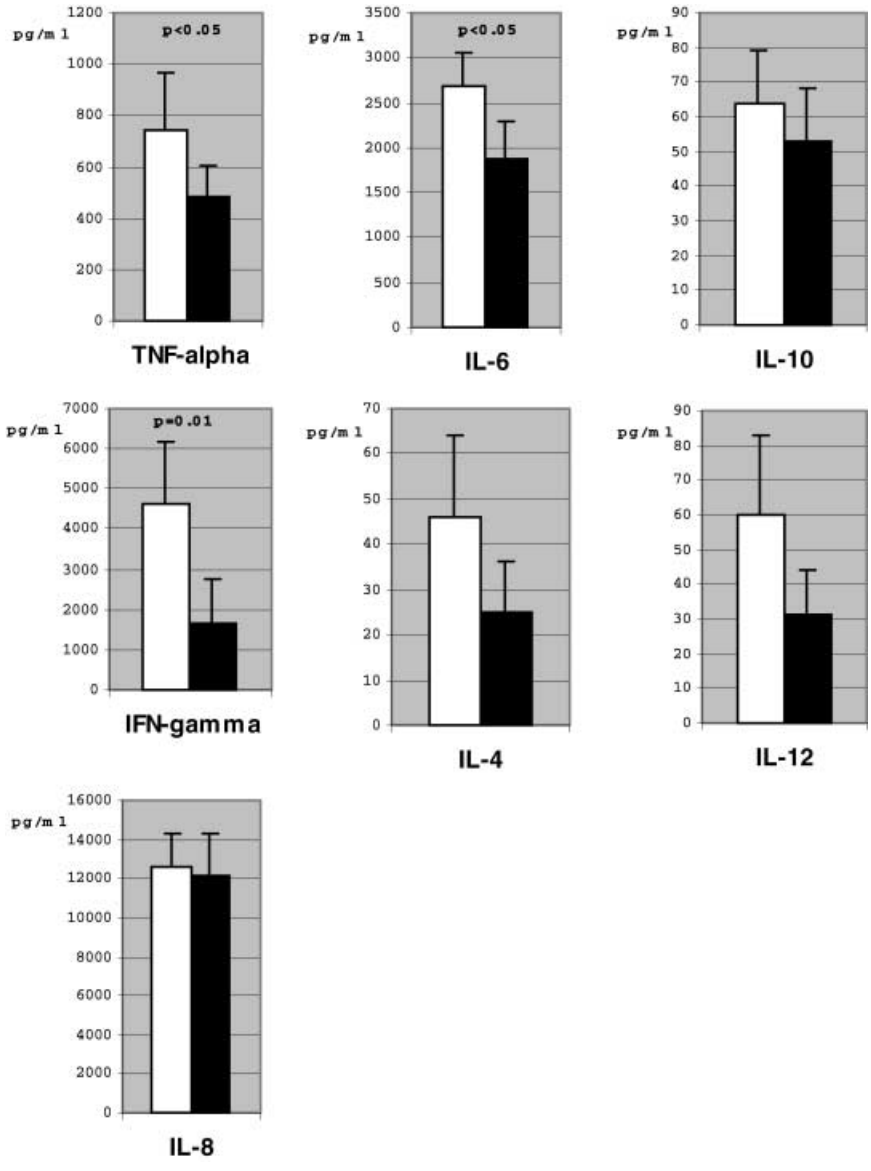

Fig. 2 Peripheral leukocyte capacity to produce cytokines in vitro (mean ±SEM) before (*open bars*) and after 2 h (*solid bars*) of mechanical ventilation

Cellular composition of blood

We observed significant changes in the cellular composition of the whole-blood samples. There was a increase in the percentage of granulocytes ($p<0.05$) and a decrease in the percentage of lymphocytes ($p<0.05$; Table 1). The percentage of CD3 and CD4 increased slightly but significantly ($p<0.05$). The percentage of CD16/56 tended to decrease (Table 1).

Table 1 Cellular composition of the whole blood samples (mean percentage ±SEM) before and after 2 h of mechanical ventilation

	Before	After	*p*
Granulocytes	45.7±4.8	56.1±5.5	<0.05
Lymphocytes	49.7±5.0	38.9±5.4	<0.05
Monocytes	4.3±0.4	4.0±0.8	n.s.
CD3	57.4±2.7	61.0±3.4	<0.05
CD4	35.1±3.0	38.5±3.0	<0.05
CD8	24.7±1.6	25.2±2.1	n.s.
CD19	22.0±2.5	21.2±2.4	n.s.
CD16/56	15.5±1.9	12.3±2.1	0.05

NK cell activity

As a result of 2 h of mechanical ventilation the killing capacity of NK cells to lyse K562 target cells decreased significantly ($p<0.01$). The mean percentage of activity decreased from 35.1±5.1 to 22.3±4.3. This remarkable decrease in killing capacity of NK cells cannot be explained by a decrease in the total numbers of NK cells (Table 1).

Cortisol

Serum cortisol levels measured before and after mechanical ventilation were similar, 0.29±0.11 µmol/l and 0.28±0.16 µmol/l, respectively

Discussion

The major finding of the present study is that exposing infants with normal lung function to 2 h of volatile anesthetics, mechanical ventilation, and cardiac catheterization is associated with remarkable changes in immune responses. We observed a proinflammatory response in the lungs with a significant increase in TNF-α, while anti-inflammatory cytokine concentrations in tracheal aspirates remained virtually unchanged, just above the detection level. In addition, the functional capacity of peripheral blood leukocytes to produce proinflammatory cytokines in vitro was significantly decreased, in particular IFN-γ, TNF-α, and IL-6. This was accompanied by a significant decrease in the activity of NK cells. This indicates that this procedure is associated with a change in the Th1/Th2 balance with a decreased Th1 immune response.

A major question from our study is which aspect of the total procedure consisting of exposure to volatile anesthetics, ventilation, and catheterization is responsible for the observed changes in the inflammatory response of our patients. A recent review article summarized the effect of anesthetic agents on the immune response and concluded that there is little evidence to support the concept of clinically relevant immune modulation by anesthetics during major surgery [23]. No clinical study has examined the effect of servoflurane on the immune response in infants and young children. Experimental studies have shown that during mechanical ventilation of uninjured lungs several volatile anesthetics may augment gene expression of proinflammatory cytokines in rat alveolar macrophages [24]. However, servoflurane was not associated with a significant increase in gene expression of proinflammatory cytokines or with concentrations of TNF-α in the lavage fluid of these rats over that with mechanical ventilation alone [24]. Kotani et al. [25] demonstrated in mechanically ventilated adult patients that intravenous propofol or volatile isoflurane produced a similar increase in gene expression of all proinflammatory cytokines on alveolar macrophages. This is remarkable since the route of administration of these anesthetics are completely different. One would have expected that by directly acting on alveolar macrophages the volatile anesthetic – isoflurane – would induce faster and probably more pronounced gene expression. It therefore remains questionable whether these clinical observed effects are all attributable to general anesthesia.

Any effect of anesthesia is likely to be overwhelmed by the neuroendocrine stress response during major surgery [23]. However, in our study the response of the hypothalamo-pituitary-adrenal axis to the catheterization procedure is probably negligible. Serum cortisol levels measured before and after mechanical ventilation were similar. Other factors such as hemorrhage, hypotension, ischemia/reperfusion, and blood transfusion, which may affect immune competence during major surgery, were negligible in our study. Thus the catheterization procedure can therefore not considered to be major surgery.

We are therefore left with the possibility that the changes in the immune response in our study were the result of mechanical ventilation, although we are aware that definitive conclusions cannot be made. Several experimental studies have reported that injurious ventilatory strategies increase TNF-α mRNA expression and lung lavage levels of TNF-α protein [7, 9, 11]. In these studies tidal volumes were very high (40 ml/kg), and/or there was preexisting lung injury. Pretreatment with intratracheal instillation of anti-TNF-α antibodies improved oxygenation, reduced infiltration of leukocytes, and ameliorated pathological findings [26]. The results of the experimental studies clearly demonstrated that TNF-α plays a pivotal role in initiating an inflammatory cascade induced by mechanical ventilation. The lung macrophage may be the critical mechanosensor cell capable of producing TNF-α in response to stretching mechanical forces [27], although there is evidence that the pulmonary epithelium may also be a key player in this regard [28].

It is remarkable, however, that such a significant proinflammatory response was observed with the ventilatory strategy we adopted. Our patients had normal lungs, and a tidal volume of 10 ml/kg should not cause overdistention, since the patients would not have the marked heterogeneities in pulmonary compliance that exist in patients with ARDS [29, 30]. This is supported by the observation that peak inspiratory pressures remained low (19.1±2.0 cmH_2O) throughout the 2-h period. To our knowledge, only one other study has examined the effect of mechanical ventilation on release of cytokines in patients with normal lung function [31]. Wrigge et al. [31] observed that after 1 h of mechanical ventilation plasma levels of pro- and anti-inflammatory mediators remained low and did not differ from baseline. Unfortunately, the local production of cytokines in the lung was not measured. The observed effects in our study may be explained by a two-hit hypothesis in which any one factor by itself does not induce an effect, but a combination of factors act synergistically to cause the changes in immune response, i.e., mechanical ventilation and volatile anesthetics.

It remains speculative what causes the onset of the peripheral immune response. One of the mechanisms could be that TNF-α produced locally in the lung causes leukocyte redistribution from the systemic circulation into the alveolar space [9, 11]. Mechanical ventilation may

recruit T cell subsets with distinctive properties with respect to homing and trafficking into inflamed sites [32]. We observed that the functional capacity of peripheral blood leukocytes to produce proinflammatory cytokines in vitro was significantly decreased, in particular INF-γ, TNF-α, and IL-6. IFN-γ is associated with a Th1 response, which is considered to be beneficial in terms of an appropriate and effective response to various stimuli, including trauma, infection, and perhaps mechanical ventilation [2]. IFN-γ is also important in stimulating the cytolytic activity of NK cells and $CD8^+$ cytotoxic T lymphocytes. The decrease in IFN-γ production was also accompanied by a significant decrease in the killing activity of NK cells. The altered Th1/Th2 balance in favor of Th2 cytokine activity may be a systemic adaptation to the proinflammatory milieu in the lung.

In conclusion, 2 h of servoflurane and mechanical ventilation with a tidal volume of 10 ml/kg is associated with remarkable changes in the immune response in infants without preexisting lung pathology undergoing cardiac catheterization. In the lungs a proinflammatory response pattern dominates without detectable concentrations of anti-inflammatory mediators. We observed a decrease in the Th1 immune response by peripheral blood leukocytes. The altered Th1/Th2 balance in favor of Th2 cytokine activity may be a systemic adaptation to the proinflammatory milieu in the lung. Further studies possibly using different anesthetic agents, different operative procedures, and different ventilatory strategies are needed to establish the mechanisms and clinical relevance of our findings.

Acknowledgements The authors thank the pediatric cardiologists and cardioanesthetists for their technical assistance.

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