Anesthesiology 2009; 110:1316–26

Anesthetic-induced Improvement of the Inflammatory Response to One-lung Ventilation

Elisena De Conno, M.D., Marc P. Steurer, M.D.,* Moritz Wittlinger, M.D.,† Marco P. Zalunardo, M.D.,‡ Walter Weder, M.D.,§ Didier Schneiter, M.D.,‖ Ralph C. Schimmer, M.D.,# Richard Klaghofer, Ph.D.,** Thomas A. Neff, M.D.,* Edith R. Schmid, M.D.,†† Donat R. Spahn, M.D.,‡‡ Birgit Roth Z'graggen, Ph.D.,§§ Martin Urner, M.D.,† Beatrice Beck-Schimmer, M.D.‖‖*

Background: **Although one-lung ventilation (OLV) has become an established procedure during thoracic surgery, sparse data exist about inflammatory alterations in the deflated, reventilated lung. The aim of this study was to prospectively investigate the effect of OLV on the pulmonary inflammatory response and to assess possible immunomodulatory effects of the anesthetics propofol and sevoflurane.**

Methods: **Fifty-four adults undergoing thoracic surgery with OLV were randomly assigned to receive either anesthesia with intravenously applied propofol or the volatile anesthetic sevoflurane. A bronchoalveolar lavage was performed before and after OLV on the lung side undergoing surgery. Inflammatory mediators (tumor necrosis factor α, interleukin 1β, interleukin 6, interleukin 8, monocyte chemoattractant protein 1) and cells were analyzed in lavage fluid as the primary endpoint. The clinical outcome determined by postoperative adverse events was assessed as the secondary endpoint.**

Results: **The increase of inflammatory mediators on OLV was significantly less pronounced in the sevoflurane group. No difference in neutrophil recruitment was found between the groups. A positive correlation between neutrophils and mediators was demonstrated in the propofol group, whereas this correlation was missing in the sevoflurane group. The number of composite adverse events was significantly lower in the sevoflurane group.**

Conclusions: **This prospective, randomized clinical study suggests an immunomodulatory role for the volatile anesthetic sevoflurane in patients undergoing OLV for thoracic surgery with significant reduction of inflammatory mediators and a significantly better clinical outcome (defined by postoperative adverse events) during sevoflurane anesthesia.**

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 9A.

ONE-LUNG ventilation (OLV) has become a standard procedure for many interventions in thoracic surgery with a need for deflation of the lung to facilitate the surgical procedure. Experimental and clinical studies have shown that mechanical ventilation with increased tidal volume and airway pressure can induce a proinflammatory reaction in the nondeflated, ventilated lung.[1–3] However, only limited data exist on inflammatory alterations in the temporarily deflated, nonventilated and thus atelectatic lung in patients undergoing thoracic surgery.

Several studies have shown that airway epithelial cells express and secrete various immune molecules such as adhesion molecules (intercellular adhesion molecule 1 [ICAM-1]), cytokines (tumor necrosis factor α [TNF-α], interleukin 1β [IL-1β], interleukin 6 [IL-6], interleukin 8 [IL-8]), and chemokines (cytokine-induced neutrophil chemoattractant 1, monocyte chemoattractant protein 1 [MCP-1]).[4–6] TNF-α, IL-1β, IL-6, IL-8, and obviously also MCP-1 are important chemoattractants that are responsible for recruitment of effector cells such as neutrophils and alveolar macrophages.[7,8] Through the expression and production of these inflammatory mediators, the airway epithelium is thought to play an important role in the initiation and exacerbation of an inflammatory response within the airways.

Atelectasis is a major cause for alveolar hypoxia. Funakoshi *et al.*[9] demonstrated increased whole-lung cytokine production in a rabbit model of short-term atelectasis, followed by reventilation. However, no direct measurements upon atelectasis were performed without assessing the effect of reventilation. Another study focused on the inflammatory reaction in alveolar macrophages in the airway compartment during alveolar hypoxia.[10] OLV was performed for 60 min. Immediately after OLV, alveolar macrophages were harvested and analyzed in culture. Cells from the deflated, nonventilated lung showed increased IL-1 and TNF levels compared with the nondeflated, ventilated control lung. These results illustrated for the first time the consequences of atelectasis without evaluation of changes due to reventilation. However, no studies have been performed so far elucidating this problem in a clinical setting.

Volatile anesthetics, such as sevoflurane and isoflurane, have been shown to attenuate cardiac injury upon

* Senior Research Assistant and Senior Physician, † Research Assistant and Resident, ‖‖ Professor, Institute of Anesthesiology, University Hospital, Zurich Institute of Physiology and Zurich Center for Integrative Human Physiology, University of Zurich, Switzerland. ‡ Senior Research Assistant and Senior Physician, ‡‡ Professor, Institute of Anesthesiology, § Professor, ‖ Senior Research Assistant and Senior Physician, Division of Thoracic Surgery, # Senior Research Assistant and Senior Physician, Department of Surgery, ** Senior Research Assistant, Department of Psychosocial Medicine, †† Professor, Division of Cardiac Anesthesia, Institute of Anesthesiology, University Hospital, Zurich, Switzerland. §§ Senior Research Assistant, Institute of Physiology and Zurich Center for Integrative Human Physiology, University of Zurich.

Received from the Institute of Anesthesiology, University of Zurich, Zurich, Switzerland. Submitted for publication June 28, 2008. Accepted for publication January 20, 2009. Supported by grant No. 3200B0-109558 from the Swiss National Science Foundations, Berne, Switzerland; Abbott AG, Baar, Switzerland; Theodor und Ida Herzog-Egli Stiftung, Zollikon, Switzerland, Von Tobel Private Bank Stiftung, Zurich, Switzerland; Lungenliga, Zurich, Switzerland; and Jubiläumsstiftung der Schweizerischen Lebensversicherungs-und Rentenanstalt, Zurich, Switzerland. Drs. De Conno and Steurer contributed equally to this work.

Address correspondence to Dr. Beck-Schimmer: Institute of Anesthesiology, Institute of Physiology and Zurich Center for Integrative Human Physiology, University of Zurich, Rämistrasse 100, Hof E 111, CH-8091 Zurich, Switzerland. beatrice.beck@usz.ch, beatrice_beck.schimmer@access.uzh.ch.

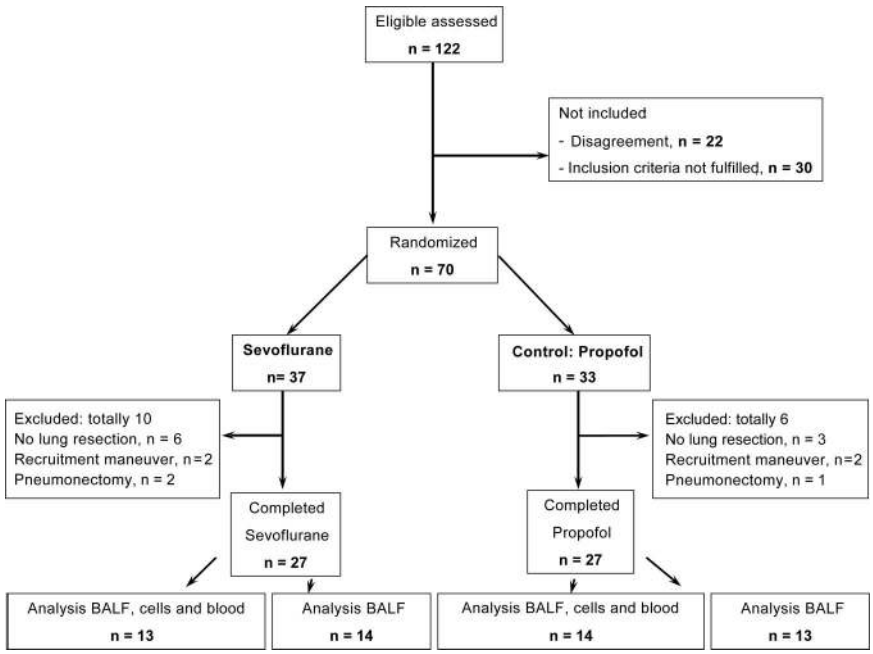

Fig. 1. Enrollment and treatment. BALF = bronchoalveolar lavage fluid.

ischemia–reperfusion.[11] Lee *et al.*[12] recently described direct antiinflammatory and antinecrotic effects of sevoflurane in cultured human kidney cells after injury. Sevoflurane attenuated expression of inflammatory mediators as well as neutrophil accumulation in a model of endotoxin-induced injury in alveolar epithelial cells (AECs) *in vitro*.[13] In addition, Reuterhaus *et al.*[14] observed an antiinflammatory effect of isoflurane in a model of endotoxin-induced lung injury *in vivo*. Finally, a recent study in patients indicated an immunomodulatory effect of the volatile anesthetic desflurane in the nondeflated, ventilated lung with high tidal volumes during OLV.[15]

The objective of this prospective, randomized clinical study was to elucidate the impact of OLV on the pulmonary inflammatory response in the atelectatic lung and to assess possible immunomodulatory effects of the volatile anesthetic sevoflurane under these conditions. Increase of inflammatory mediators in bronchoalveolar lavage fluid (BALF) was determined as the primary endpoint, and postoperative complications were determined as the secondary endpoints. The research hypothesis was that sevoflurane attenuates hypoxia (OLV)–induced pulmonary inflammation assessed by decreased production of inflammatory mediators in the nonventilated, deflated lung.

Materials and Methods

The study was approved by the institutional review board (Zurich, Switzerland) for human studies and internationally registered at ClinicalTrials.gov (NCT00515905). The manuscript complies with the Consolidated Standards of Reporting Trials requirements. Written informed patient consent was obtained from all participants. We studied 54 adults with American Society of Anesthesiologists physical status I–III, scheduled to undergo elective thoracic surgery with lung resection performed through thoracotomy or thoracoscopy, and requiring OLV during surgery (fig. 1). Patients were randomly assigned to receive either total intravenous anesthesia with propofol (propofol group, n = 27) or inhalation anesthesia with sevoflurane (sevoflurane group, n = 27).

Exclusion criteria were ongoing treatment with any dose of systemic or topical steroids, acute pulmonary or extrapulmonary infections (elevated C-reactive protein [CRP] > 10 ng/ml [reference range < 5 ng/ml] or leukocytosis $> 10 \times 10^3/\mu l$ [reference range $3.0–9.6 \times 10^3/\mu l$]), severe chronic obstructive pulmonary disease (Gold stage 2–4), history of recurrent pneumothoraces, pneumonectomy, and/or lung volume–reduction surgery.

Anesthesia

All patients received premedication with 7.5 mg oral midazolam 1 h before the induction of anesthesia. In patients undergoing thoracotomy and lacking general contraindications for regional anesthesia, a thoracic epidural catheter was inserted at T4–T5 to T7–T8 for intraoperative and postoperative pain management and was started at a continuous infusion rate of 5–8 ml/h with 0.33% ropivacaine.

Induction of anesthesia was initiated with the intravenous anesthetic propofol and a target-controlled infusion technique in the propofol group with target concentrations of 3–5 μg/ml or with propofol administered as bolus (1.5–2.5 mg/kg) in the sevoflurane group. Tracheal intubation was facilitated using fentanyl (3 μg/kg) and atracurium (0.5 mg/kg) in both groups. Maintenance of

anesthesia was performed with either propofol target-controlled infusion with 1 minimum alveolar concentration (MAC)-awake (age adjusted) or with sevoflurane (1 MAC, age adjusted) according to randomization. Intraoperative pain management was continued with intravenous application of fentanyl in 1- to 2-μg/kg boluses according to clinical needs and with continuous epidural infusion of 0.33% ropivacaine (5–8 ml/h) in patients with thoracic epidural anesthesia. In addition, remifentanil at 0.1–0.3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ was used. For further muscle relaxation, atracurium (bolus administrations of 10 mg) was applied as clinically required.

In both groups, a left- or right-sided double-lumen endobronchial tube (37–41 Ch; Pharmaceutiques Rüsch, Moissy Cramayel, France) was inserted, and the correct position was confirmed using a fiberoptic bronchoscope (Intubation Fiberscope LF-2; Medical Systems and Endoscopes, Olympus Europe, Volketswil, Switzerland). Pressure-controlled ventilation with 5 cm H_2O positive end-expiratory pressure and peak pressure less than 30 cm H_2O was used for both double-lung ventilation and OLV. The inspiratory oxygen fraction (F_{IO_2}) was set to 0.8 during double-lung ventilation and increased to 1.0 during OLV or performance of bronchoalveolar lavage (BAL). For double-lung ventilation, tidal volumes up to 8 ml/kg and a respiratory frequency of 10–15/min were chosen to maintain arterial carbon dioxide tension between 35–45 mmHg. For OLV, tidal volumes up to 6–7 ml/kg with a respiratory frequency of 10–20/min were used with an F_{IO_2} of 1.0 to ensure an oxygen saturation greater than 85%. Patients requiring insufflation of oxygen, recruitment maneuver, or even intermittent continuous positive airway pressure on the deflated, nonventilated lung were excluded from the analysis because they did not fulfill the criteria for inclusion in the intention-to-treat population (OLV and alveolar hypoxia without reoxygenation [two patients in each group]).

After completion of surgery, reventilation of the previously nonventilated lung was performed (manual inflation for 10 s to 30 cm H_2O four times), and after discontinuation of anesthetic agents, patients were extubated and taken directly to a recovery room or the intensive care unit for postanesthetic care.

During anesthesia, invasive blood pressure measurements, electrocardiogram, heart rate, oxygen saturation, end-tidal carbon dioxide, central venous pressure, body temperature, and diuresis were continuously monitored and recorded in both groups. Arterial blood gas analysis was performed as required.

Crystalloids were used for hydration. Hydroxyethyl starch (130/0.4) and additional crystalloids were given according to clinical needs. None of the patients received blood transfusions. A perioperative antibiotic prophylaxis was given to all patients.

Primary Endpoints

BALF and Plasma. Primary endpoints were inflammatory mediators in BALF. The first BAL was performed before OLV at the later deflated, nonventilated lung undergoing surgery (T1), and the second BAL was performed immediately after reexpansion and reventilation of the same lung at the end of surgery (T2). At the same time points, T1 and T2, 10 ml peripheral blood was collected from the arterial catheter (BD Vacutainer Systems, Plymouth, United Kingdom) from the last 27 patients (14 patients from the propofol group and 13 patients from the sevoflurane group) (fig. 1). In addition, cells in the BALF of these patients were determined quantitatively and qualitatively.

In all patients, BAL was performed by flexible fiberoptic bronchoscopy under sterile conditions using sodium chloride 0.9% in an average of 150 ml per BAL. The tip of the bronchoscope was wedged into a subsegment bronchus of the lung. The chosen segment was lavaged after instillation of 50 ml sterile saline solution (0.9%, pH 7.4); afterwards the lavage fluid was gently aspirated. This procedure was repeated two times in different subsegments. The recovery rate of the lavage fluid was approximately 50%.

Both BALF and blood samples were centrifuged (BALF: 10°C, 5 min at 1,500 rpm; blood: 10°C, 10 min at 2,000 rpm), and supernatants of BALF as well as plasma were stored at −20°C. Cell pellets from centrifuged BALF from 27 patients were assessed for differential cell counts using cytospins and Diff-Quick (Dade Behring, Duedingen, Switzerland). The difference of neutrophils at T2 minus T1 was calculated in percentages and analyzed in correlation to the difference in the concentration of inflammatory mediators in BALF.

For analysis of human inflammatory mediators in BALF and plasma (TNF-α, IL-1β, IL-6, IL-8, MCP-1), enzyme-linked immunosorbent assays were used (TNF-α: BD Biosciences Pharmingen, San Diego, CA; other enzyme-linked immunosorbent assays: R&D Systems, Minneapolis, MN).

Chemotaxis Assays. Human neutrophils from healthy donors were prepared by centrifugation through Ficoll gradients as described before.[16] Purified neutrophils were labeled with 10 μM carboxylfluorescein diacetate succinimidyl ester for 15 min at 37°C (CFDA-SE; Invitrogen AG, Basel, Switzerland). Cells were washed twice with 0.9% NaCl and resuspended in Ham's 12 medium, 1 mM 4-(2- hydroxyethyl)-1-piperazineethanesulfonic acid, containing a 1:10 dilution of 10% gelatin in H_2O, pH 7.2. After isolation, cells were allowed to adhere to glass coverslips for 15 min at 37°C. The coverslips were then rinsed and placed on Zigmond chambers.[17] Aliquots (100 μl) of Dulbecco's modified Eagle's Medium (Invitrogen AG), supplemented with 1% fetal bovine serum, were added to one side of the chamber, and the same solution containing *N*-formyl-methyl-leucyl-

phenylalanine (10^{-5} M) was added to the other side as a positive control. The same experiments were performed with 100 μl BALF from different patients on both sides of the chamber, whereby one patient of the propofol group was matched to one patient from the sevoflurane group with similar OLV time (six matched pairs were tested).

Time-lapsed videomicroscopy was used to examine neutrophil movements in Zigmond chambers. Neutrophils were recorded moving along a chemoattractant gradient in the chamber. The microscope was equipped with differential interference contrast optics and a 10× objective. Images were captured at 1-min intervals for 2 h with an LX Widefield high-speed and highly sensitive microscope for live cell imaging (Leica, Solms, Germany). The system is built on an inverted microscope with fast filter wheels for excitation from ultraviolet to visible.

The recorded data were analyzed using the spot tracking algorithm of Imaris Software (Bitplane AG, Zurich, Switzerland). Displacement vectors were construed from the position of the track start to the position of the track end. The normalized displacement vectors were visualized in a scatterplot. The averaged displacement direction was calculated by taking the first principal component of the set of all direction vectors, *i.e.*, the unit length vector v that maximizes sum (<v,d_i>^2), where d_i denotes the (normalized) displacement vectors. It was then plotted as a superimposed arrow. For quantitative analysis of movement, we calculated the counterclockwise angles in radians between the x-axis and the displacement vectors in two-dimensional euclidean space. The number of cells migrating in a specific direction (represented by the calculated angle of the displacement vector) was plotted as a histogram.

Secondary Endpoints

The clinical postoperative outcome was assessed as secondary endpoint based on adverse events. The following parameters were determined: CRP preoperatively (baseline), on postoperative day 1 (POD1), postoperative day 3 (POD3), and postoperative day 5 (POD5). Also, leukocyte count in the blood of the patients was registered at the same time points.

The following adverse events were defined during the postoperative course: pulmonary infections necessitating antibiotic treatment, radiographically diagnosed pneumonia, radiographically diagnosed atelectasis, radiographically diagnosed effusion, fistula, reintubation, systemic inflammatory response syndrome, sepsis, acute respiratory distress syndrome, surgical revision, and death.

Statistical Analysis

The intention-to-treat population comprised all patients with OLV and alveolar hypoxia without reoxygenation. This intention-to-treat population was used for the primary analysis. The sample size calculation was performed based on the estimate of a 40% difference in expression on inflammatory mediators at a 5% level of statistical significance and a power of 80% (propofol group: 500 ± 300 [mean ± SD]; sevoflurane group: 300 ± 200; assumption from *in vitro* data only). Based on these calculations, 27 patients were required for each study group. To compensate for withdrawals, 70 patients were enrolled in the study.

All analyses were performed with the statistical software program SPSS (SPSS Inc., Chicago, IL), release 12.0. Descriptive data are shown as median and interquartile range, mean and SD, or count and percentage.

Differences between the propofol group and the sevoflurane group were tested by the Mann–Whitney U test. The Spearman rho was used to assess the associations between the variables. Comparisons between groups were controlled for type I error rate using the Bonferroni correction for multiple comparisons: $\alpha' = \alpha$:m, where α' is the corrected level of significance, α is the unadjusted level of significance, and m is the number of comparisons.

Because of a significant difference in OLV time between the two groups, comparisons were adjusted using OLV time as a covariate and multivariate analysis of variance. Because the assumptions for this statistical procedure were not consistently met by the data characteristics, the results should be interpreted with caution. The outcomes of this procedure are therefore reported in parentheses and in a descriptive manner.

A *P* value less than 0.05 was considered to be significant.

Results

Patient Characteristics and Surgical Criteria

A total of 54 patients were enrolled to the study with wedge resections as well as partial and total lobectomies. None of these patients showed any signs of preoperative infection. There were no differences in patient characteristics and surgical data between the two groups, except for a longer duration of OLV in the propofol group (140 ± 76 min) *versus* the sevoflurane group (98 ± 57 min) ($P < 0.05$; table 1). The range of applied doses of fentanyl per patient was 0.2–4 mg in the propofol group and 0.2–3.8 mg in the sevoflurane group. Similarly, no difference was found for remifentanil (0–5.1 *vs.* 0–4.7 mg). In the propofol group, patients received between 400 and 3,700 ml crystalloids (600–3,400 ml in the sevoflurane group) and between 0 and 1,700 ml hydroxyethyl starch (0–1,500 ml in the sevoflurane group), with no statistically significant difference between groups.

Table 1. Patient Characteristics and Surgical Data

	Propofol Group n = 27	Sevoflurane Group n = 27
Sex, M/F	18/9	14/13
Age, yr	58 ± 12	55 ± 15
BMI, kg/m^2	26 ± 5	25 ± 4
Diagnosis, malignancies/structure	20/7	20/7
Thoracotomy/thoracoscopy	22/5	19/8
Duration of surgery, min	181 ± 91	154 ± 71
Duration of anesthesia, min	288 ± 102	260 ± 82
OLV duration, min	140 ± 76	98 ± 57*

Data are expressed as absolute numbers in mean ± SD. Malignancies: carcinomas, metastases. Structure: fibrotic tumors, bronchiectasis.

* $P < 0.05$.

BMI = body mass index; OLV = one-lung ventilation.

Inflammatory Mediator Expression after OLV

One-lung ventilation resulted in an increase in the measured inflammatory mediators in both the propofol and the sevoflurane groups (calculated as difference in concentrations of inflammatory mediators in BALF, performed after OLV at T2 and before OLV at T1). However, the increase of inflammatory mediators upon OLV in the propofol group was significantly higher for all parameters except IL-1β compared with the sevoflurane group ($P < 0.05$; table 2 and figs. 2A–E).

The magnitude of cytokine expression also varied over the course of OLV (figs. 3A–E). A progressive increase in TNF-α, IL-6, IL-8, MCP-1, and to a lesser degree IL-1β was observed with increasing duration of OLV. For all parameters except IL-1β, this increase was again smaller in patients with sevoflurane anesthesia. Interestingly, the cytokine increase in the propofol group seemed to follow a linear pattern up to approximately 120 min of OLV, whereas thereafter the magnitude of the expression increased to a larger degree, approaching an exponential fashion (figs. 3C and D). This was also observed in patients receiving sevoflurane; however, again a substantial attenuation compared with the propofol group was apparent.

Cellular Response to OLV

One-lung ventilation resulted in an increase in polymorphonuclear cells in BALF in both the propofol and the sevoflurane groups by approximately 10%. Interestingly, the increase in the inflammatory mediators IL-1β, IL-8, IL-6, and MCP-1 showed a significant correlation with accumulation of polymorphonuclear cells (determined as the percentage of neutrophils in the lavage at T2 minus the percentage of neutrophils in the lavage at T1) in the propofol group but not in the sevoflurane group. For TNF-α no correlation between neutrophil accumulation and mediator expression was observed for both groups (fig. 4A), whereas significant correlations in the propofol group were found for IL-1β ($r = 0.281$, $P < 0.05$; fig. 4B), IL-6 ($r = 0.512$, $P < 0.05$; fig. 4C), and IL-8 ($r = 0.466$, $P < 0.01$; fig. 4D). However, in the sevoflurane group, such correlations were missing (IL-1β: $r = 0.024$, not significant [NS]; IL-6: $r = 0.091$, NS; IL-8: $r = 0.116$, NS). Between neutrophils and MCP-1, also a known neutrophil chemoattractant, no correlation was observed in either group ($r = 0.157$ and $r = 0.009$, NS; fig. 4E). While the lack of correlation in the sevoflurane group compared with the propofol group might also reflect a consequence of the reduced inflammatory response in this group, the differences between the correlations in the propofol group *versus* the sevoflurane group did not reach statistical significance.

To underline the biologic relevance of the measured inflammatory mediators, BALF samples of a propofol–sevoflurane pair of patients with similar OLV times were tested for chemotaxis activity. As shown in figure 5, neutrophils were attracted by the BALF of the propofol patient (fig. 5A). Figures 5B and C reflect the positive control experiment with the *N*-formyl-methyl-leucyl-phenylalanine.

Clinical Evaluation

C-reactive protein and blood leukocyte count as additional parameters for inflammation were assessed preoperatively, on POD1, POD3, and POD5, to determine possible differences in the sevoflurane compared with the propofol group. No statistically significant difference in CRP and leukocyte count could be detected between the anesthesia groups from POD1 to POD5 (table 3). Interestingly, as seen in table 4, there was a significant correlation between CRP values and OLV time on POD1 in the propofol group ($r = 419$, $P < 0.05$), which was

Table 2. Expression of Inflammatory Mediators after OLV

	Propofol Median (IQR)	Sevoflurane Median (IQR)	*P* Value*	Propofol Mean ± SD	Sevoflurane Mean ± SD	*P* Value†
TNF-α	0.9 (1.5)	0.0 (0.4)	< 0.05	1.37 ± 1.78	0.41 ± 1.05	NS
IL-1β	1.5 (20.8)	1.4 (3.9)	NS	10.96 ± 18.12	5.47 ± 11.31	NS
IL-6	37.0 (159)	9.0 (25.0)	< 0.05	93.77 ± 117.5	28.89 ± 43.87	0.05
IL-8	117.0 (782)	26.0 (88.0)	< 0.05	486.04 ± 700.03	62.19 ± 85.2	0.008
MCP-1	97.8 (212.5)	23.1 (47.0)	< 0.05	130.23 ± 122.62	55.36 ± 87.04	0.048

* *P* value after Bonferroni correction. † *P* value in multivariate analysis of variance (covariate: one-lung ventilation [OLV] time).

IL-1β = interleukin 1β; IL-6 = interleukin 6; IL-8 = interleukin 8; IQR = interquartile range; MCP-1 = monocyte chemoattractant protein 1; NS = not significant; TNF-α = tumor necrosis factor α.

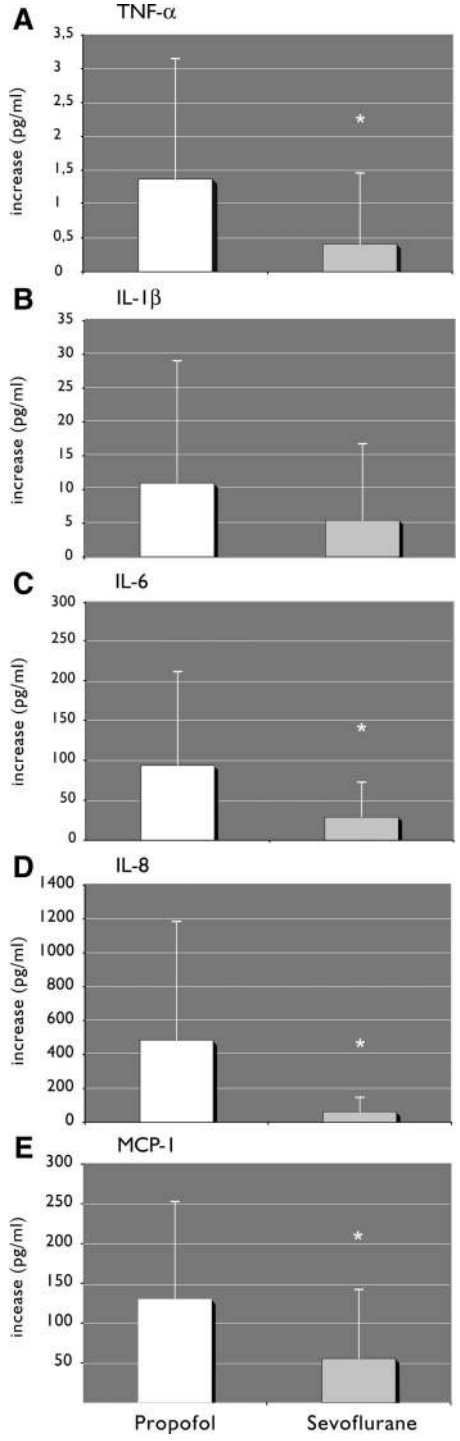

Fig. 2. (*A*) Change of tumor necrosis factor-α (TNF-α) concentration in bronchoalveolar lavage upon one-lung ventilation in the propofol and sevoflurane group. * $P < 0.001$. (*B*) Change of interleukin-1β (IL-1β) concentration in bronchoalveolar lavage upon one-lung ventilation in the propofol and sevoflurane group. Not significant. (*C*) Change of interleukin-6 (IL-6) concentration in bronchoalveolar lavage upon one-lung ventilation in the propofol and sevoflurane group. * $P < 0.05$. (*D*) Change of interleukin-8 (IL-8) concentration in bronchoalveolar lavage upon one-lung ventilation in the propofol and sevoflurane group. * $P < 0.005$. (*E*) Change of monocyte chemoattractant protein-1 (MCP-1) concentration in bronchoalveolar lavage upon one-lung ventilation in the propofol and sevoflurane group. * $P < 0.05$. Values are mean ± SD.

decreased and not significant in the sevoflurane group ($r = 0.226$, NS).

To obtain information about a possible secondary systemic inflammatory reaction about OLV, inflammatory mediators were determined in plasma before and immediately after OLV, and differences (T2 minus T1) were calculated. While plasma TNF-α, IL-1β, and IL-8 were not detectible at both time points, plasma concentrations of IL-6 and MCP-1 were increased after OLV. We therefore correlated values from these mediators to CRP values on POD1 (table 5). A correlation of 0.459 was calculated between CRP value on POD1 and increase of plasma IL-6 concentration in the propofol group ($P < 0.05$), whereas no correlation was observed in the sevoflurane group ($r = 0.324$, NS). Similar data were found for the correlation between CRP and MCP-1 in the propofol group ($r = 0.535$, $P < 0.05$) and in the sevoflurane group ($r = 0.166$, NS).

Adverse events are reported in table 6. The overall number of adverse events in the propofol group was significantly higher than in the sevoflurane group (40 *vs.* 18; $P < 0.05$). In addition, patients in the propofol group spent significantly more time in the intensive care unit compared with patients in the sevoflurane group (1.52 ± 2.33 *vs.* 0.87 ± 0.43 days; $P < 0.05$). (*P* values are adjusted to the difference of OLV time.)

Discussion

Volatile anesthetics have been shown to have cardioprotective effects.[11,18,19] Although some studies have demonstrated protection using volatile anesthetics before the onset of injury, others also indicate that protection against injury is still achieved when administered after myocardial ischemia. Two clinical trials even showed cardioprotective effects of sevoflurane when the anesthetics were used throughout cardiac surgery.[20,21] We therefore were interested to explore potential protective benefits of sevoflurane compared with propofol anesthesia during thoracic surgery with OLV.

Injury to cells or tissues related to an inflammatory response can be ascribed to a complex array of mediators generated and released from activated phagocytes such as neutrophils and macrophages or by target cells such as AECs. Cytokines and chemokines are implicated in the recruitment of effector cells toward target tissues, whereby TNF-α and interleukins are strong neutrophil chemoattractants.

Previous studies have shown attenuating effects of volatile anesthetics in a model of AEC injury. A study in rat AECs showed that exposure to volatile anesthetics altered secretion of inflammatory mediators upon IL-1β stimulation.[22] Halothane, isoflurane, and enflurane decreased production of IL-6, macrophage inflammatory protein-2, and MCP-1 protein concentrations in a dose-

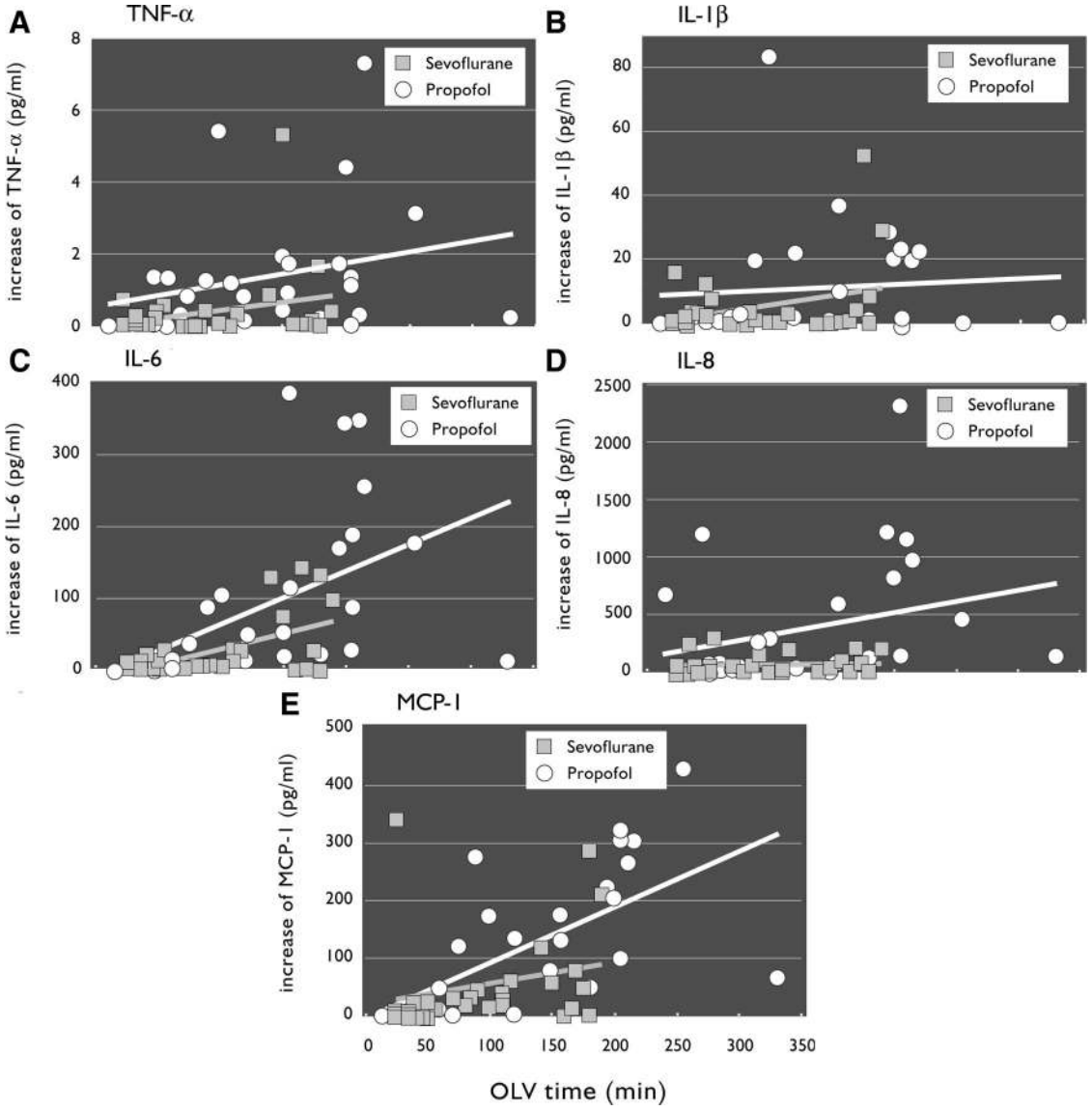

Fig. 3. (*A*) Increase of one-lung ventilation (OLV) time correlated to increase of tumor necrosis factor α (TNF-α) in bronchoalveolar lavage. Propofol: $r = 0.069$, not significant (NS); sevoflurane: $r = 0.062$, NS. (*B*) Increase of OLV time correlated to increase of interleukin 1β (IL-1β) in bronchoalveolar lavage. Propofol: $r = 0.006$, NS; sevoflurane: $r = 0.091$, NS. (*C*) Increase of OLV time correlated to increase of interleukin 6 (IL-6) in bronchoalveolar lavage. Propofol: $r = 0.227$, $P < 0.05$; sevoflurane: $r = 0.311$, $P < 0.005$. (*D*) Increase of OLV time correlated to increase of interleukin 8 (IL-8) in bronchoalveolar lavage. Propofol: $r = 0.069$, NS; sevoflurane: $r = 0.005$, NS. (*E*) Increase of OLV time correlated to increase of monocyte chemoattractant protein 1 (MCP-1) in bronchoalveolar lavage. Propofol: $r = 0.362$, $P < 0.001$; sevoflurane: $r = 0.058$, NS.

and time-dependent manner. AECs are a potent source of inflammatory mediators within the respiratory compartment of the lung upon injury with endotoxins.[23] Our group has therefore tested the effect of sevoflurane in an *in vitro* model of AEC stimulation. In a first step, AECs were preexposed to sevoflurane, followed by stimulation with endotoxin.[13] Results of this study demonstrated decreased expression of chemokines with attenuated chemotaxis. Exposing AECs first to endotoxin with a sevoflurane treatment after the onset of injury resulted again in attenuation of the inflammatory reaction.[16]

In the current clinical trial, the production of the corresponding inflammatory mediators TNF-α, IL-1β, IL-6, IL-8, and MCP-1 in the respiratory compartment was quantitatively assessed upon OLV for thoracic surgery. It is shown that the increase of these mediators is diminished in the sevoflurane group compared with the propofol group. Based on these results, it seems that in the propofol group a significantly more pronounced inflammatory reaction is present. This clinical study suggests that the use of sevoflurane might have the potential to reduce the AEC-induced inflammatory answer in OLV for thoracic surgery. Chemotaxis assays underlined the biologic relevance of these observations.

Because we were also interested in assessing the biologic consequences of this change of expression of me-

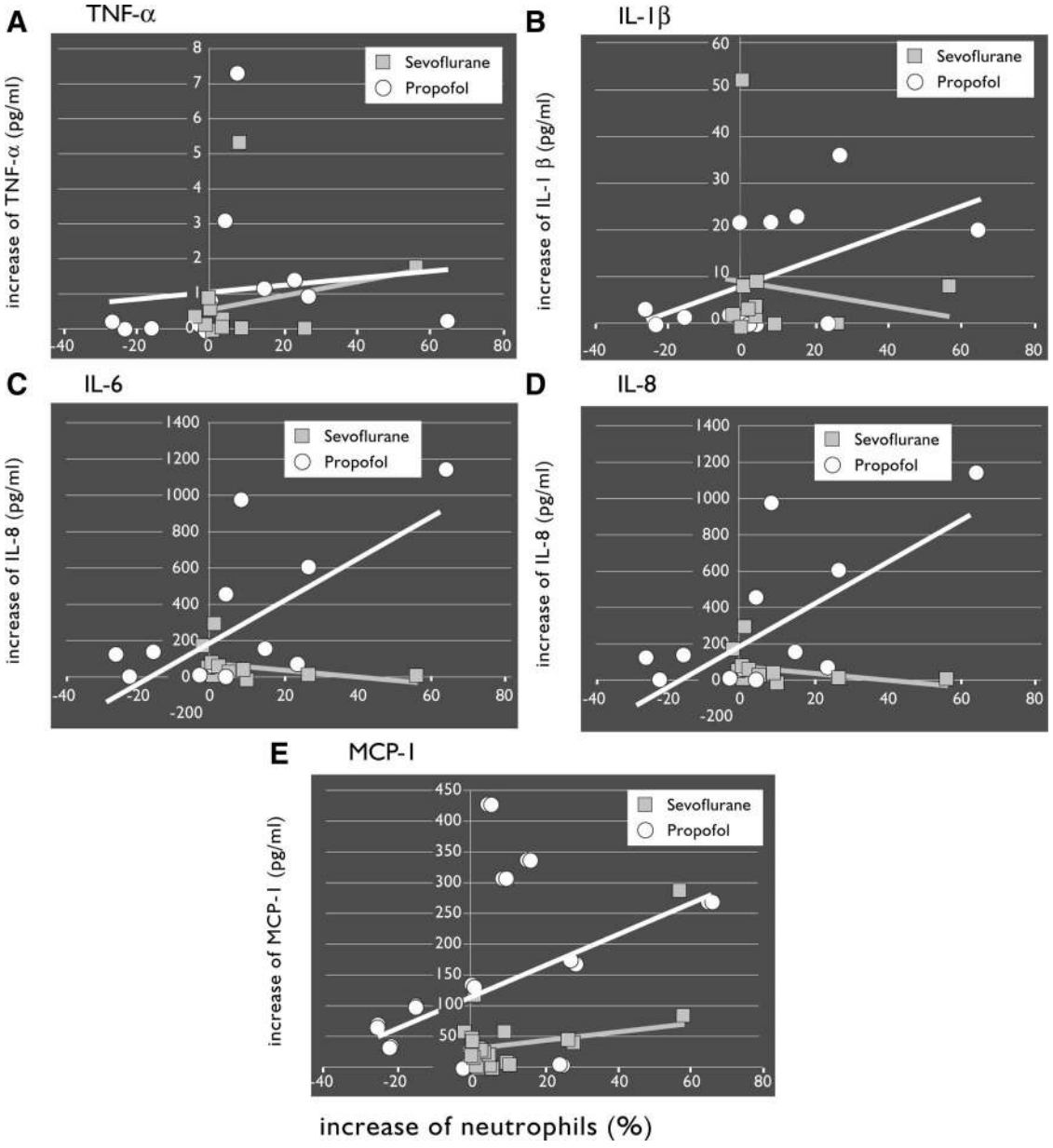

Fig. 4. (*A*) Increase of neutrophil content (%) in bronchoalveolar lavage correlated to increase of tumor necrosis factor α (TNF-α). Propofol: $r = 0.013$, not significant (NS); sevoflurane: $r = 0.049$, NS. (*B*) Increase of neutrophil content (%) in bronchoalveolar lavage correlated to increase of interleukin 1β (IL-β). Propofol: $r = 0.281$, $P < 0.05$; sevoflurane: $r = 0.024$, NS. (*C*) Increase of neutrophil content (%) in bronchoalveolar lavage correlated to increase of interleukin 6 (IL-6). Propofol: $r = 0.512$, $P < 0.05$; sevoflurane: $r = 0.091$, NS. (*D*) Increase of neutrophil content (%) in bronchoalveolar lavage correlated to increase of interleukin 8 (IL-8). Propofol: $r = 0.466$, $P < 0.01$; sevoflurane: $r = 0.116$, NS. (*E*) Increase of neutrophil content (%) in bronchoalveolar lavage correlated to increase of monocyte chemoattractant protein 1 (MCP-1). Propofol: $r = 0.157$, NS; sevoflurane: $r = 0.103$, NS.

diators on a cellular level, we quantified neutrophils in BALF and possible relations between cell influx and up-regulation of mediators. Although MCP-1 is well known as a macrophage chemoattractant, it might also be responsible for neutrophil recruitment under certain conditions.[7,8] The analyses showed a significant correlation of the increase in polymorphonuclear cells and the up-regulation of IL-1β, IL-6, and IL-8 in the propofol group, but not for TNF-α and MCP-1. During sevoflurane anesthesia, however, the inflammatory reaction, assessed by the magnitude of cytokine expression, was significantly attenuated and not correlated with the increase of polymorphonuclear cells in BALF. These results point at a reduced inflammatory response at molecular and cellular level during sevoflurane anesthesia.

A major finding of this study was the improved outcome for patients in the sevoflurane group, which showed approximately 50% fewer adverse events compared with the propofol patients. Because our analysis had shown an almost exponential increase of inflammatory mediators in correlation to OLV time in the propofol group, we postulated a biologic phenomenon of this

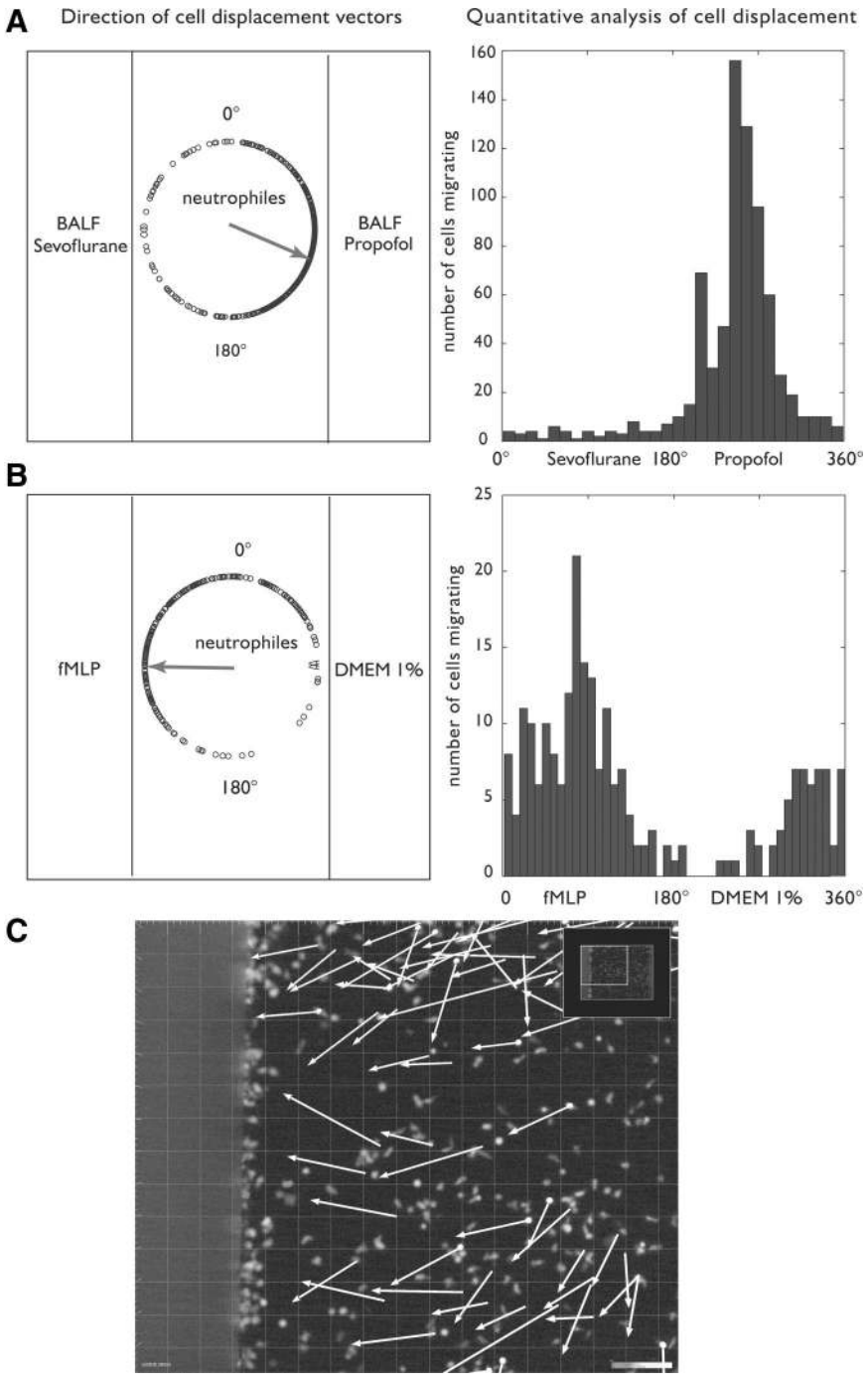

Fig. 5. (*A*) Chemotaxis assay performed in Zigmond chamber. Displacement of fluorescein-labeled human neutrophils was assessed and calculated after exposure to bronchoalveolar lavage fluid of a patient from the propofol group (*right*) and from the sevoflurane group (*left*) with similar one-lung ventilation time. The figure represents one of six similar migration pictures. (*B*) Chemotaxis assay performed in Zigmond chamber. Displacement of fluorescein-labeled human neutrophils was assessed and calculated after exposure to control medium (Dulbecco's modified Eagle's Medium [DMEM]/1% fetal bovine serum [FBS]) and the chemoattractant formyl-Met-Leu-Phe (fMLP). The figure represents one of four similar migration pictures. (*C*) Chemotaxis assay performed in Zigmond chamber. Displacement of fluorescein-labeled human neutrophils was documented upon exposure to control medium (DMEM/1% FBS) (*right*) and the chemoattractant fMLP (*left*), using time-lapsed videomicroscopy. The figure represents one of four similar migration pictures. BALF = bronchoalveolar lavage fluid.

observation and therefore evaluated CRP and blood leukocyte count. Interestingly, a significant correlation was observed between CRP and OLV time in the propofol group and was clearly attenuated in the sevoflurane group. Also, CRP values on POD1 were correlated with IL-6 and MCP-1 during propofol anesthesia, whereas during sevoflurane anesthesia the correlation was less accentuated and not significant. These data might point out a potential link between inflammatory mediators in the lung and outcome.

Lung injury after thoracic surgery is relatively uncommon, but it is a major complication with high mortality.[24,25] A recent study showed a combined frequency of acute lung injury and acute respiratory distress syndrome of 3.9% with a mortality of 72% for patients developing acute respiratory distress syndrome.[26] Several factors need to be considered as possible triggers for development of acute lung injury in OLV for thoracic surgery: (1) During OLV, the operated lung remains completely atelectatic for a period of time, in parallel with hypoperfusion due to hypoxic vasoconstriction. Lung reexpansion and tissue reperfusion might reflect an ischemia–reperfusion injury, which could explain the underlying mechanism of inflammation. (2) The postresectional re-

Table 3. C-reactive Protein and Leukocyte Count in Blood Samples

	Propofol Group, n = 27	Sevoflurane Group, n = 27
CRP, ng/ml		
BL	9 ± 5	5 ± 6
POD1	67 ± 36	74 ± 47
POD3	135 ± 73	125 ± 86
POD5	75 ± 73	66 ± 51
Leukocytes, count × $10^3/\mu l$		
BL	7.5 ± 2.2	6.7 ± 2.5
POD1	9.9 ± 2.8	8.3 ± 2.5
POD3	9.1 ± 2.4	7.7 ± 2.9
POD5	9.2 ± 1.9	7.3 ± 2.2

Data are expressed as absolute numbers in mean ± SD.

BL = baseline; CRP = C-reactive protein; POD1 = postoperative day 1; POD3 = postoperative day 3; POD5 = postoperative day 5.

maining tissue has been subjected to considerable mechanical manipulation during the conducted lobectomy or segmentectomy, also contributing to an inflammatory response. (3) Ventilation of the ventilated lung with a high inspiratory oxygen concentration during OLV could lead to an injury of both the ventilated and the collapsed lung. (4) Mechanical ventilation itself can cause damage to the ventilated lung. The mechanisms are still not clear, and the evidence for their involvement in acute lung injury after OLV is questionable.[24,27,28] (5) Reexpansion of the collapsed lung induces lung injury with increased microvascular permeability leading to reexpansion pulmonary edema.[29,30]

During OLV, the deflated, alveolar hypoxia occurs in the nonventilated lung. Recently, the involvement of AECs in hypoxia-induced lung injury has been determined *in vitro*.[31] Hypoxia leads to enhanced expression of adhesion molecules on AECs with increased neutrophil adherence, demonstrating that the lower respiratory epithelial compartment might play an important role in inflammatory mechanisms during hypoxia-induced lung injury. In the current study, reventilation/reperfusion injury is rather unlikely because the lavage was performed immediately upon reventilation. Therefore, besides the aforementioned possible mechanisms (1–5), hypoxia could be added as a possible trigger inducing inflammatory reactions (6).

It is well known that upon OLV hypoxic vasoconstriction occurs in this lung to reduce ventilation/perfusion mismatch. The effect of this vasoconstriction on lung

Table 4. Correlation between CRP on Postoperative Day 1 and OLV Time

			OLV Time
Propofol	CRP	Correlation coefficient	0.419
		Significance	0.030
Sevoflurane	CRP	Correlation coefficient	0.226
		Significance	0.277

CRP = C-reactive protein; OLV = one-lung ventilation.

Table 5. Correlation between CRP on Postoperative Day 1 and Plasma IL-6 and MCP-1

			IL-6	MCP-1
Propofol	CRP	Correlation coefficient	0.459	0.535
		Significance	0.050	0.022
Sevoflurane	CRP	Correlation coefficient	0.324	0.166
		Significance	0.189	0.510

CRP = C-reactive protein; IL-6 = interleukin 6; MCP-1 = monocyte chemoattractant protein 1.

parenchyma is unclear; theoretically, it results in consecutive tissue hypoxia. In a recent study focusing on lung injury upon OLV,[32] the authors investigated the vascular compartment after 60 min of OLV in pigs and showed congested vasculature in the deflated, nonventilated lung. These findings from the vasculature, together with our data from the respiratory tract, would support the idea of compartmentalized inflammatory reactions. Mulligan *et al.*[33] defined a compartmentalized role for inflammatory mediators in a model of intrapulmonary deposition of immunoglobulin G immune complexes. In addition, sevoflurane-induced attenuation of hypoxic pulmonary vasoconstriction itself might also influence the degree of injury.

The duration of OLV seems to be essential, as previously shown by Tekinbas *et al.*[34] This group randomly allocated rats into groups with different OLV periods. Lung tissue myeloperoxidase activity, defined as a parameter for neutrophils, increased in parallel with OLV time. Also, histopathologic findings such as alveolar edema and infiltration of lung tissue with inflammatory cells were in accordance with the OLV time. These findings were also supported by Misthos *et al.*,[35] showing that magnitude of oxidative stress was related to OLV duration. Our data might support these findings.

While Schilling *et al.* showed a beneficial effect of desflurane on the nondeflated, ventilated lung during OLV, this is the first study focusing on the possible

Table 6. Adverse Events

	Events	
	Propofol	Sevoflurane
Prolonged antibiosis	7	4
Pneumonia	3	2
Atelectasis	5	1
Fistula	3	1
Effusion	15	10
Reintubation	1	0
SIRS	1	0
Sepsis	1	0
ARDS	0	0
Surgical revision	4	0
Death	0	0
Total	40	18

ARDS = acute respiratory distress syndrome; SIRS = systemic inflammatory response syndrome.

benefit of the volatile anesthetic on the deflated, nonventilated lung.[15]

In summary, this prospective, randomized study reveals an immunomodulatory role for the volatile anesthetic sevoflurane in patients undergoing OLV for thoracic surgery, demonstrating a possible antiinflammatory effect. An important finding of the current study is that the sevoflurane group showed not only an attenuated inflammatory reaction at the organ level in the lung undergoing OLV, but also an improved postoperative course with significantly lower overall number of adverse events.

The authors thank Irene Odermatt (Art Designer, Institute of Anesthesiology, University of Zurich, Switzerland) for development of illustrations.

References

1. Dreyfuss D, Saumon G: Ventilator-induced lung injury: Lessons from experimental studies. Am J Respir Crit Care Med 1998; 157:294–323
2. Dreyfuss D, Saumon G: From ventilator-induced lung injury to multiple organ dysfunction? Intensive Care Med 1998; 24:102–4
3. Schilling T, Kozian A, Huth C, Buhling F, Kretzschmar M, Welte T, Hachenberg T: The pulmonary immune effects of mechanical ventilation in patients undergoing thoracic surgery. Anesth Analg 2005; 101:957–65
4. Madjdpour C, Oertli B, Ziegler U, Bonvini JM, Pasch T, Beck-Schimmer B: Lipopolysaccharide induces functional ICAM-1 expression in rat alveolar epithelial cells *in vitro*. Am J Physiol Lung Cell Mol Physiol 2000; 278:L572–9
5. Simon RH, Paine R III: Participation of pulmonary alveolar epithelial cells in lung inflammation. J Lab Clin Med 1995; 126:108–18
6. Takizawa H: Airway epithelial cells as regulators of airway inflammation. Int J Mol Med 1998; 1:367–78
7. Vozzelli MA, Mason SN, Whorton MH, Auten RL Jr: Antimacrophage chemokine treatment prevents neutrophil and macrophage influx in hyperoxia-exposed newborn rat lung. Am J Physiol Lung Cell Mol Physiol 2004; 286: L488–93
8. Beck-Schimmer B, Schwendener R, Pasch T, Reyes L, Booy C, Schimmer RC: Alveolar macrophages regulate neutrophil recruitment in endotoxin-induced lung injury. Respir Res 2005; 6:61–6
9. Funakoshi T, Ishibe Y, Okazaki N, Miura K, Liu R, Nagai S, Minami Y: Effect of re-expansion after short-period lung collapse on pulmonary capillary permeability and pro-inflammatory cytokine gene expression in isolated rabbit lungs. Br J Anaesth 2004; 92:558–63
10. Kisala JM, Ayala A, Stephan RN, Chaudry IH: A model of pulmonary atelectasis in rats: Activation of alveolar macrophage and cytokine release. Am J Physiol 1993; 264:R610–4
11. Tanaka K, Ludwig LM, Kersten JR, Pagel PS, Warltier DC: Mechanisms of cardioprotection by volatile anesthetics. ANESTHESIOLOGY 2004; 100:707–21
12. Lee HT, Kim M, Jan M, Emala CW: Anti-inflammatory and antinecrotic effects of the volatile anesthetic sevoflurane in kidney proximal tubule cells. Am J Physiol Renal Physiol 2006; 291:F67–78
13. Suter D, Spahn DR, Blumenthal S, Reyes L, Booy C, Z'Graggen BR, Beck-Schimmer B: The immunomodulatory effect of sevoflurane in endotoxin-injured alveolar epithelial cells. Anesth Analg 2007; 104:638–45
14. Reutershan J, Chang D, Hayes JK, Ley K: Protective effects of isoflurane pretreatment in endotoxin-induced lung injury. ANESTHESIOLOGY 2006; 104:511–7
15. Schilling T, Kozian A, Kretzschmar M, Huth C, Welte T, Buhling F, Hedenstierna G, Hachenberg T: Effects of propofol and desflurane anaesthesia on the alveolar inflammatory response to one-lung ventilation. Br J Anaesth 2007; 99:368–75
16. Yue T, Roth Z'graggen B, Blumenthal S, Neff SB, Reyes L, Booy C, Steurer M, Spahn DR, Neff TA, Schmid ER, Beck-Schimmer B: Postconditioning with a volatile anaesthetic in alveolar epithelial cells *in vitro*. Eur Respir J 2008; 31: 118–25
17. Zigmond SH: Ability of polymorphonuclear leukocytes to orient in gradients of chemotactic factors. J Cell Biol 1977; 75:606–16
18. Kato R, Foex P: Myocardial protection by anesthetic agents against ischemia-reperfusion injury: An update for anesthesiologists. Can J Anaesth 2002; 49:777–91
19. Mullenheim J, Ebel D, Frassdorf J, Preckel B, Thamer V, Schlack W: Isoflurane preconditions myocardium against infarction *via* release of free radicals. ANESTHESIOLOGY 2002; 96:934–40
20. De Hert SG, Van der Linden PJ, Cromheecke S, Meeus R, Nelis A, Van Reeth V, ten Broecke PW, De Blier IG, Stockman BA, Rodrigus IE: Cardioprotective properties of sevoflurane in patients undergoing coronary surgery with cardiopulmonary bypass are related to the modalities of its administration. ANESTHESIOLOGY 2004; 101:299–310
21. Jakobsen CJ, Berg H, Hindsholm KB, Faddy N, Sloth E: The influence of propofol *versus* sevoflurane anesthesia on outcome in 10,535 cardiac surgical procedures. J Cardiothorac Vasc Anesth 2007; 21:664–71
22. Giraud O, Molliex S, Rolland C, Lecon-Malas V, Desmonts JM, Aubier M, Dehoux M: Halogenated anesthetics reduce interleukin-1β–induced cytokine secretion by rat alveolar type II cells in primary culture. ANESTHESIOLOGY 2003; 98:74–81
23. dos Santos CC, Han B, Andrade CF, Bai X, Uhlig S, Hubmayr R, Tsang M, Lodyga M, Keshavjee S, Slutsky AS, Liu M: DNA microarray analysis of gene expression in alveolar epithelial cells in response to TNFα, LPS, and cyclic stretch. Physiol Genomics 2004; 19:331–42
24. Baudouin SV: Lung injury after thoracotomy. Br J Anaesth 2003; 91:132–42
25. Grichnik KP, D'Amico TA: Acute lung injury and acute respiratory distress syndrome after pulmonary resection. Semin Cardiothorac Vasc Anesth 2004; 8:317–34
26. Kutlu CA, Williams EA, Evans TW, Pastorino U, Goldstraw P: Acute lung injury and acute respiratory distress syndrome after pulmonary resection. Ann Thorac Surg 2000; 69:376–80
27. Jordan S, Mitchell JA, Quinlan GJ, Goldstraw P, Evans TW: The pathogenesis of lung injury following pulmonary resection. Eur Respir J 2000; 15:790–9
28. Gothard J: Lung injury after thoracic surgery and one-lung ventilation. Curr Opin Anaesthesiol 2006; 19:5–10
29. Nakamura M, Fujishima S, Sawafuji M, Ishizaka A, Oguma T, Soejima K, Matsubara H, Tasaka S, Kikuchi K, Kobayashi K, Ikeda E, Sadick M, Hebert CA, Aikawa N, Kanazawa M, Yamaguchi K: Importance of interleukin-8 in the development of reexpansion lung injury in rabbits. Am J Respir Crit Care Med 2000; 161:1030–6
30. Sakao Y, Kajikawa O, Martin TR, Nakahara Y, Hadden WA III, Harmon CL, Miller EJ: Association of IL-8 and MCP-1 with the development of reexpansion pulmonary edema in rabbits. Ann Thorac Surg 2001; 71:1825–32
31. Beck-Schimmer B, Schimmer RC, Madjdpour C, Bonvini JM, Pasch T, Ward PA: Hypoxia mediates increased neutrophil and macrophage adhesiveness to alveolar epithelial cells. Am J Respir Cell Mol Biol 2001; 25:780–7
32. Yin K, Gribbin E, Emanuel S, Orndorff R, Walker J, Weese J, Fallahnejad M: Histochemical alterations in one lung ventilation. J Surg Res 2007; 137:16–20
33. Mulligan MS, Vaporciyan AA, Warner RL, Jones ML, Foreman KE, Miyasaka M, Todd RF, Ward PA: Compartmentalized role for leukocytic adhesion molecules in lung inflammatory injury. J Immunol 1994; 154:1350–63
34. Tekinbas C, Ulusoy H, Yulug E, Erol MM, Alver A, Yenilmez E, Geze S, Topbas M: One-lung ventilation: For how long? J Thorac Cardiovasc Surg 2007; 134:405–10
35. Misthos P, Katsaragakis S, Theodorou D, Milingos N, Skottis I: The degree of oxidative stress is associated with major adverse effects after lung resection: A prospective study. Eur J Cardiothorac Surg 2006; 29:591–5