The Immune Response to One-Lung Ventilation

Clinical and Experimental Studies

THOMAS SCHILLING
Dissertation presented at Uppsala University to be publicly examined in Enghoffsalen, Akademiska sjukhuset, 751 85 UPPSALA, Ing. 50, Thursday, December 10, 2009 at 09:15 for the degree of Doctor of Philosophy in Medicine. The examination will be conducted in English.

Abstract

One-lung ventilation (OLV) as an established procedure during thoracic surgery may be injurious in terms of increased mechanical stress characterised by alveolar cell stretch and overdistension, increased cyclic tidal recruitment of alveolar units, compression of alveolar vessels and increased pulmonary vascular resistance. This may result in ventilation-induced lung injury with pro-inflammatory cytokine production, leukocyte recruitment and neutrophil-dependent tissue destruction.

Despite the consequences of delivering the whole tidal volume ($V_T$) to only a single lung, relatively high $V_T$ are used during OLV to maintain arterial oxygenation and carbon dioxide elimination. However, this may increase mechanical stress in the dependent lung and may aggravate alveolar injury.

There is a lack of data on the alveolar immune consequences of OLV. Therefore, the present studies investigate the epithelial damage and pro-inflammatory response induced by mechanical ventilation and OLV. OLV induced pulmonary injury, but alveolar damage in the ventilated lung decreased by reduction of the tidal volume in patients scheduled for thoracic surgery (study I). The use of the volatile anaesthetic desflurane in OLV patients attenuated the OLV-induced alveolar immune response (study II).

Furthermore, an experimental model of thoracic surgery was established to investigate the systemic and pulmonary consequences of OLV and thoracic surgery in comparison with the effects of conventional two-lung ventilation and spontaneous breathing. The experimental data indicate that beside the pulmonary immune response volatile anaesthetics have also modulated the plasma concentrations of cytokines during and after OLV (study III). In contrast, OLV and thoracic surgery increased the expression of pro-inflammatory mRNA in BAL cells and lung tissue samples. General anaesthesia did not affect this response (study IV).

The results of the present studies indicate that OLV and thoracic surgery may be injurious to the lung tissue to a similar degree. The recruitment and activation of alveolar granulocytes characterise the alveolar damage. The administration of different anaesthetics modulates the activation of alveolar cells, specified by decreased inflammatory mediator release in subjects that receive desflurane anaesthesia, which does not affect the expression of cytokine mRNA in alveolar cells and lung tissue samples.

**Keywords:** One-lung ventilation, open thoracic surgery, ventilation-induced lung injury, alveolar immune response, bronchoalveolar lavage, propofol, desflurane, cytokines, animal model, mRNA, RT-PCR

*Thomas Schilling, Clinical Physiology, Akademiska sjukhuset, Uppsala University, SE-75185 Uppsala, Sweden*

© Thomas Schilling 2009

ISSN 1651-6206
urn:nbn:se:uu:diva-108851 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-108851)
To my parents

Dorothee and Dr. Ulrich Schilling
List of Publications

The thesis is based on the following publications, which are referred to in the text using the Roman numerals I-IV.


Reprints were made with permission granted by the publishers Lippincott Williams & Wilkins and Oxford University Press, respectively.
Abbreviations

ALI  Acute Lung Injury
ARDS  Acute Respiratory Distress Syndrome
ARM  Alveolar Recruitment Manoeuvre
ASA  American Society of Anesthesiologists
BAL  Bronchoalveolar Lavage
BALF  Bronchoalveolar Lavage Fluid
CI  Cardiac Index
CVP  Central Venous Pressure
DAD  Diffuse Alveolar Damage
DLT  Double-Lumen Tube
DO$_2$i  Oxygen Delivery Index
ECG  Electrocardiogram
etCO$_2$  End-Tidal Carbon Dioxide
EVLW  Extra-Vascular Lung Water
FEV$_1$  Forced Expiratory Volume in one Second
FiO$_2$  Fraction of Inspired Oxygen
GEDV  Global End-Diastolic Volume
HR  Heart Rate
ID  Inner Diameter
IL  Interleukin
IPPV  Intermittent Positive Pressure Ventilation
IQR  Interquartile Range
ITBV  Intrathoracic Blood Volume
MAC  Minimal Alveolar Concentration
MAP  Mean Arterial Pressure
MCP-1  Monocyte-Chemoattractant Protein-1
MIP-2  Macrophage-Inflammatory Protein-2
MPAP  Mean Pulmonary Arterial Pressure
mRNA  Messenger Ribonucleic Acid
MV  Minute Ventilation
NYHA  New York Heart Association
OLV  One-Lung Ventilation
PAC  Pulmonary Artery Catheter
paO$_2$  Partial Pressure of Oxygen in Arterial Blood
paCO$_2$  Partial Pressure of Carbon Dioxide in Arterial Blood
PAOP  Pulmonary Artery Occlusion Pressure
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{AW}$</td>
<td>Airway Pressure</td>
</tr>
<tr>
<td>PEEP</td>
<td>Positive End-Expiratory Pressure</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorph-Nuclear Granulocytes</td>
</tr>
<tr>
<td>PPE</td>
<td>Post-Pneumonectomy Pulmonary Edema</td>
</tr>
<tr>
<td>PVR</td>
<td>Pulmonary Vascular Resistance</td>
</tr>
<tr>
<td>Qs/Qt</td>
<td>Intrapulmonary Shunt (Venous Admixture)</td>
</tr>
<tr>
<td>RR</td>
<td>Respiratory Rate</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse Transcriptase Polymerase Chain Reaction</td>
</tr>
<tr>
<td>SaO$_2$</td>
<td>Arterial Oxygen Saturation</td>
</tr>
<tr>
<td>sICAM</td>
<td>soluble Intercellular Adhesion Molecule</td>
</tr>
<tr>
<td>SVR</td>
<td>Systemic Vascular Resistance</td>
</tr>
<tr>
<td>SvO$_2$</td>
<td>Mixed venous Oxygen Saturation</td>
</tr>
<tr>
<td>TIVA</td>
<td>Total Intravenous Anaesthesia</td>
</tr>
<tr>
<td>TLV</td>
<td>Two-Lung Ventilation</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour Necrosis factor</td>
</tr>
<tr>
<td>$V_T$</td>
<td>Tidal Volume</td>
</tr>
<tr>
<td>VC</td>
<td>Vital Capacity</td>
</tr>
<tr>
<td>VILI</td>
<td>Ventilation-Induced Lung Injury</td>
</tr>
<tr>
<td>V/Q</td>
<td>Ventilation / Perfusion relationship</td>
</tr>
<tr>
<td>VO$_{2i}$</td>
<td>Oxygen Consumption Index</td>
</tr>
</tbody>
</table>
INTRODUCTION

Clinical background

The patient scheduled to undergo open thoracic surgery is at risk of increased perioperative respiratory morbidity and mortality [1-2]. Lung injury has been recognised as a complication of lung resection since Zeldin et al. reported ten cases of acute lung injury following thoracic surgery and introduced the term “post-pneumonectomy pulmonary edema” (PPE) in 1984 [3]. However, PPE is not limited to pneumonectomy but may also occur after lobectomy and minor procedures with lower incidence and better outcome.

In the comprehensive form the clinical course of PPE is indistinguishable from the Acute Respiratory Distress Syndrome (ARDS) as defined by the American-European Consensus Conference on ARDS [4]. However, many patients do not meet the diagnostic criteria for ARDS but fulfil those of the less severe acute lung injury (ALI). Hence, the reported incidence for PPE differs with rates of 4-7% for pneumonectomy and 1-7% for lobectomy but the syndrome has a high mortality of 40-70% [5]. Indeed, ALI/ARDS is the major cause of death in the respective patient group after thoracic surgery [6].

Pathogenesis of lung injury following thoracic surgery

Acute pro-inflammatory responses become evident in all types of thoracic surgery [5], which are well described in relation to ALI/ARDS [7]. The lung damage after thoracic surgery probably represents the pulmonary manifestations of an inflammatory injury. The loss of endothelial integrity leads to changes in permeability, to the loss of high amounts of protein into the alveolar space and to characteristic histological changes. These histomorphological changes are equivalent, if not identical to those of ARDS.

Consequently, histological analysis of diffuse alveolar damage in a porcine model of thoracic surgery [8] established considerable alveolar and interstitial oedema, neutrophil infiltration, alveolar overdistension, microhaemorrhage and microatelectasis in the temporarily collapsed,
surgically manipulated lung and also in the dependent ventilated lung [9]. The detection of a diffusely distributed alveolar damage in both lungs indicates that mechanical ventilation may be as harmful as a period of complete lung collapse and manipulation during thoracic surgery.

Important risk factors for postoperative ALI following lung resection include surgery-related conditions such as duration and invasiveness of the procedure [10-12], the extent of lung resection [13] and right-sided thoracotomy [14]. Impaired lymphatic drainage and fluid overload were also identified as corroborating the deleterious effect of mechanical ventilation.

The mechanism of lung injury produced by surgery itself is different from lung injury induced by mechanical ventilation. Surgical manipulation exerts direct effects on the lung tissue structure. This injury may be complicated by reventilation-/reperfusion-like alveolar damage [15] as observed in rabbit lungs after complete collapse and reventilation [16]. Additionally, translocation of pro-inflammatory mediators from the lung [17], induced either by surgery or by injurious ventilation is considered to be a source of systemic inflammation that in turn possibly affects the contralateral lung.

Ventilation-induced lung injury (VILI)

Mechanical two-lung ventilation produces homogeneously distributed alveolar damage itself [9] and generates an inflammatory response in the alveoli even in healthy lungs [18]. The resulting ventilation-induced lung injury (VILI) [19] is characterised by dysfunction of the surfactant system [20], alveolar and interstitial oedema [21], leukocyte recruitment [22], cytokine production [23] and neutrophil-dependent tissue destruction [24].

Different mechanisms during mechanical ventilation may lead to cellular activation and mediator release. These are in particular mechanical forces applied to the lung tissue, which may result in alveolar cell stretch and overdistension, shearing forces secondary to repeated tidal collapse and re-opening of alveolar units [25] and increased vascular shearing stresses [26-29]. As a result, experimental and clinical studies on two-lung ventilation [30-31] revealed a progressive alteration of the pulmonary immune function during anaesthesia and surgery.

One-lung ventilation

The ventilation-induced pulmonary trauma is further amplified by exclusion of the surgically treated lung from ventilation and the delivery of the entire tidal volume to the remaining lung (one-lung ventilation, OLV). Relatively
high tidal volumes (V\textsubscript{T}) and subsequently increased airway pressures during OLV are considered to be injurious [6, 32] in terms of increased mechanical stress, characterised by increased cyclic recruitment/de-recruitment during OLV [33]. As a result, OLV may aggravate the alveolar damage followed by a permeability-type pulmonary oedema with diffuse alveolar injury [34], leukocyte sequestration [22] and alveolar cytokine release [35]. OLV thus contributes to postoperative pulmonary morbidity through induction of a truly asymmetric lung injury following thoracic surgery [36]. For that reason, these patients are at increased risk of postoperative respiratory complications, which may impair the outcome [1].

However, as understanding of the underlying mechanisms of ventilation-induced lung injury evolves, the anaesthetic care for patients undergoing OLV has been questioned as well. Protective ventilation approaches during OLV including the use of smaller tidal volumes [37] with lower inspiratory pressures (volume- and pressure-limited ventilation) seem to be favourable in patients undergoing thoracotomy [38]. Furthermore, experimental data from an isolated rabbit lung model suggest that protective one-lung ventilation with tidal volumes and PEEP, set to avoid lung collapse and overdistension is able to minimise ventilation-induced lung injury [39].

In contrast, in thoracic surgical patients undergoing OLV with high and low V\textsubscript{T}, a time-dependent increase of pro-inflammatory parameters was established. The concentrations of inflammatory mediators in tracheal aspirates were not different between the two ventilator settings, and neither time course nor concentrations of pulmonary or systemic mediators differed between the patient groups [35].

The management of patients in thoracic surgery may offer opportunities for anaesthetic interventions including protective ventilation approaches and the use of volatile anaesthetics. However, there are no data on the effects of OLV in comparison to those of complete lung collapse and surgical manipulation on the alveolar and systemic immune response after administration of volatile or intravenous anaesthetics. Moreover, clinical studies would not allow the differentiation of OLV-induced alveolar injury from the immune response after conventional two-lung ventilation or during spontaneous breathing.

Therefore, the objective of the present studies was to investigate whether OLV with tidal volumes as used during two-lung ventilation alters the pulmonary immune function in patients undergoing open thoracic surgery. The hypothesis was tested that the reduction of tidal volume decreases the ventilation-associated lung inflammation during and after OLV. The second aim was to establish the modulation of the alveolar inflammatory response to OLV by volatile or intravenous anaesthetics. A porcine model was developed to evaluate the alveolar and systemic immune consequences of OLV and thoracic surgery, and to compare these effects with the immune responses of standard TLV and spontaneous breathing.
AIMS OF THE STUDIES

The objective of the studies, which this thesis comprises, was to investigate the pulmonary immune effects of mechanical ventilation and thoracic surgery in clinical observations and in an experimental porcine model.

The pro-inflammatory response induced by one-lung ventilation (OLV) contributes to the impaired respiratory function and may increase pulmonary complications in patients after thoracic surgery.

The knowledge obtained by these studies could facilitate the implementation of a lung protective ventilation approach in thoracic surgery and may contribute to improved patient care and better outcome.

The specific objectives of the four present studies were:

1. To examine whether a standard ventilation setting with a tidal volume as used during TLV results in time-dependent alterations of the alveolar immune function in patients scheduled for elective open thoracic surgery.

2. To investigate the effects of a protective ventilation approach with lower tidal volumes on the OLV-induced pro-inflammatory response in patients.

3. To establish the effects of general anaesthesia on the pulmonary inflammatory response to OLV in a clinical study.

4. To demonstrate the effects of OLV and thoracic surgery on the expression of key inflammatory cytokines, which characterise alveolar injury in a porcine model of thoracic surgery.

5. To evaluate the modulation of the alveolar and systemic inflammatory response to OLV and surgical manipulation by volatile or intravenous anaesthetics in pigs.

6. To investigate the modulation of pro-inflammatory cytokine-mRNA expression by volatile or intravenous anaesthetics in BAL cells and representative lung tissue samples of pigs after OLV and thoracic surgery.
METHODS

Study protocols

Study I

The study evaluated whether ventilation with different tidal volumes modifies the pulmonary immune function, haemodynamic variables and gas exchange in patients who underwent elective open thoracic surgery.

The patients were randomly assigned to two groups that either received a tidal volume of 10ml·kg\(^{-1}\) or 5ml·kg\(^{-1}\) throughout the observation period (TLV before OLV, OLV, TLV after OLV).

Fibre-optic bronchoalveolar lavage of the ventilated lung was performed before and immediately after OLV and 2 hours postoperatively. The cell numbers and protein concentrations as well as TNF\(\alpha\), IL8, sICAM-1, IL10 and PMN-elastase release were determined in the BAL fluid (figure 1).

Figure 1. Study protocol (study I and II).

Study II

The study investigated whether volatile or intravenous anaesthetics differentially modulate the alveolar inflammatory response to OLV.

The patients were randomly assigned to two groups that either received propofol or desflurane anaesthesia. The synthetic opioid remifentanil completed anaesthesia in both groups.
The patients were mechanically ventilated with $V_T=10\text{ml}\cdot\text{kg}^{-1}$ during two-lung ventilation and OLV. Fibre-optic bronchoalveolar lavage of the ventilated lung was performed before and after OLV, and 2 hours postoperatively (figure 1).

Alveolar cells, protein concentrations and the release of TNFα, IL8, sICAM-1, IL10 as well as PMN-elastase into the BAL fluid were assessed.

Study III

The study established the pulmonary and systemic immune effects of OLV and thoracic surgery influenced by different anaesthetic regimen in a porcine model (figure 1).

The animals were randomised to pigs that underwent OLV with propofol or desflurane anaesthesia, with conventional TLV or spontaneous breathing (SB). The SB pigs were killed after induction of anaesthesia and bronchoalveolar lavage (BAL) to obtain reference values for the alveolar immune response (figure 1).

OLV and TLV pigs were mechanically ventilated ($V_T=10\text{ml}\cdot\text{kg}^{-1}$) throughout the experiment. OLV was performed by inflation of a left-sided bronchial blocker, and a thoracic surgical procedure was simulated. BAL of both lungs was performed and blood samples were taken before, immediately after OLV and 90min thereafter. The immune effects were assessed by serum concentrations of TNFα, IL8, IL10, and alveolar protein as well as cell numbers in the BAL fluids.

Figure 2. Experimental workflow in the studies III and IV.

Study IV

The experiment investigated the effects of volatile or intravenous anaesthetics on the expression of cytokine-mRNA in BAL cells and lung
tissue samples in pigs that underwent conventional TLV or OLV and thoracic surgery.

BAL of randomly chosen segments of both lungs was performed before, immediately after OLV and 90min thereafter (figure 2). After killing the pigs, lung tissue samples were harvested from both lungs. The cells isolated from BAL fluids and tissue samples were used to assess the expression of IL1β-, IL8-, IL10- and TNFα-mRNA in response to TLV, OLV and thoracic surgery by RT-PCR.

Clinical studies

Subjects

**Study I and II:** Altogether sixty-two patients (ASA II-III) with normal lung function scheduled for elective open thoracic surgery were included into the studies. The Ethics Committee of the Otto-von-Guericke-University Magdeburg approved the study protocols and written informed consent was obtained from each patient.

Exclusion criteria were the following: cardiac failure (NYHA≥III) or relevant obstructive or restrictive pulmonary diseases (VC or FEV₁<50% of the predicted values), pulmonary hypertension (MPAP>30mmHg) and pre-existing coagulation disorders. Patients treated with immune modulators (cytostatic drugs, corticosteroids and nonsteroidal anti-inflammatory drugs, vaccination, blood products) within three months prior to surgery were not enrolled in the study. Patients with persistent tobacco abuse and with evidence of pulmonary or systemic infections (clinically defined or elevated serum C-reactive protein concentrations, leukocytosis or body temperature >37°C) were also excluded.

The preoperative evaluation comprised a complete history, physical examination, measurements of actual body weight and height, ECG, chest roentgenogram, pulmonary function tests, echocardiogram, and arterial blood gas analysis.

Perioperative management

Two hours before surgery, each patient received diazepam (0.15mg·kg⁻¹) for premedication. Preoperatively, a thoracic epidural catheter was inserted at the level of the thoracic segments Th₄/₅ to Th₆/₇. Epidural catheterisation was performed by the loss of resistance technique via the median approach. The catheter was advanced cranially 2-3cm into the epidural space and a test dose (3ml bupivacaine, 0.5% with epinephrine, 5μg·ml⁻¹) was given to
preclude an intrathecal or intravascular position of the catheter. Epidural analgesia was commenced after OLV with ropivacaine 0.2% and sufentanil (1μg·ml⁻¹) and was maintained for 2-4 days until the chest tubes were removed.

Intraoperative fluid therapy included crystalloid and colloid infusions (7ml·kg⁻¹·h⁻¹ and 5ml·kg⁻¹, respectively). For prophylactic antibiosis, the patients received a single dose of cefotiam (2g).

After surgery, all patients were admitted to the intensive care unit and monitored for at least 24h. During postoperative stabilisation, fluids and blood transfusions were given in order to maintain central venous pressure ≥3cmH₂O, urine output ≥1ml·kg⁻¹·h⁻¹ and haemoglobin concentration ≥6.0mmol·l⁻¹. A chest X-ray examination was done in the ICU prior to extubation. After extubation, supplemental oxygen was given in order to keep peripheral oxyhaemoglobin saturation >95%. Postoperatively, all patients were assessed on a daily basis in order to detect clinical signs of pulmonary complications.

Patient anaesthesia

**Study I:** General anaesthesia was induced intravenously with propofol (1-1.5mg·kg⁻¹), cis-atracurium (0.1mg·kg⁻¹) and continuous infusion of remifentanil (0.3μg·kg⁻¹·min⁻¹). Anaesthesia was maintained by continuous infusions of propofol (4.0mg·kg⁻¹·h⁻¹), remifentanil (0.2μg·kg⁻¹·min⁻¹) and cis-atracurium (2μg·kg⁻¹·min⁻¹).

**Study II:** The patients were randomly assigned to propofol (propofol group, n=15) or desflurane (desflurane group, n=15) anaesthesia. In the propofol group, general anaesthesia was induced with propofol (1.5-2mg·kg⁻¹) and remifentanil (0.25μg·kg⁻¹·min⁻¹). Tracheal intubation was facilitated by administration of cis-atracurium (0.1mg·kg⁻¹). Anaesthesia was maintained with a continuous infusion of propofol (2-4mg·kg⁻¹·h⁻¹), remifentanil (0.1-0.4μg·kg⁻¹·min⁻¹) and cis-atracurium (2μg·kg⁻¹·min⁻¹).

In the desflurane group, anaesthesia was induced as above described but maintained with desflurane (∼1MAC), remifentanil (0.1-0.35μg·kg⁻¹·min⁻¹) and cis-atracurium (2μg·kg⁻¹·min⁻¹).

Ventilation management

After tracheal intubation with a left- or right-sided standard double-lumen tube (DLT, Broncho-Cath® 39 or 41 Charrière, Mallinckrodt Medical Ltd., Ireland), the patients were mechanically ventilated with intermittent positive pressure ventilation (IPPV, FiO₂ ∼0.45 in air, PEEP 3mmHg, respiratory rate adjusted to maintain a normal paCO₂). The correct position of the DLT was
confirmed by fibre-optic bronchoscopy. Mechanical ventilation was provided through an anaesthesia ventilator that was connected to a circle system (Cicero®, Dräger, Germany).

**Study I:** The patients were randomly assigned to two groups (random numbers generated by EXCEL®, Microsoft Corp., USA): group A (n=16) received two-lung ventilation (TLV) with a tidal volume of \( \text{V}_{\text{T}} = 10\text{ml} \cdot \text{kg}^{-1} \); group B (n=16) received TLV with reduced \( \text{V}_{\text{T}} = 5\text{ml} \cdot \text{kg}^{-1} \), related to the actual body weight. During OLV, the nondependent lung collapsed completely. The ventilatory settings were as follows: \( \text{V}_{\text{T}} \) remained unchanged at \( 10\text{ml} \cdot \text{kg}^{-1} \) and \( 5\text{ml} \cdot \text{kg}^{-1} \) respectively. Respiratory rate was adjusted to maintain a paCO\(_2\) of 35-45mmHg. FiO\(_2\) was set to 0.8-1.0 to achieve paO\(_2\) >80mmHg. Positive end-expiratory pressure (PEEP) was set to zero during OLV while avoiding peak inspiratory pressures >30mmHg and gas trapping at end-expiration. In three patients of group A, the threshold of 30mmHg was reached. The tidal volume was reduced by 1ml·kg\(^{-1}\) in these cases. End-expiratory PEEP records (0-2mmHg) revealed no differences between both groups.

**Study II:** The patients were ventilated with a constant \( \text{V}_{\text{T}} \) of \( 10\text{ml} \cdot \text{kg}^{-1} \) according to the actual body weight, FiO\(_2\) of 0.45 in air, PEEP 5cmH\(_2\)O, and the respiratory frequency adjusted to maintain paCO\(_2\) between 36 to 44mmHg. \( \text{V}_{\text{T}} = 10\text{ml} \cdot \text{kg}^{-1} \) and FiO\(_2\) = 0.8-1.0 were used during OLV to achieve paO\(_2\) >80mmHg, and RR adjusted to maintain paCO\(_2\) (~40mmHg). PEEP was set to zero during OLV. The peak inspiratory pressures were limited to 35cmH\(_2\)O.

**Postoperative ventilation management:** For postoperative respiratory support the endotracheal tubes were changed to standard single-lumen tubes (ID 8.5 or 9mm, Mallinckrodt Medical Ltd., Ireland) at the end of surgery. All patients were extubated 1-3 hours after the last bronchoscopy.

**Haemodynamic and ventilation measurements**

All patients received an arterial catheter for continuous blood pressure measurements and arterial blood sampling (20G Angiocath, Becton-Dickinson, Germany) and a pulmonary arterial catheter (Swan-Ganz thermodilution catheter, B. Braun, Germany) for cardiac output measurements (Hewlett Packard monitor M1106C, Philips, Germany) and sampling of mixed venous blood. After sampling, blood gas analysis was performed immediately with standard blood gas electrodes (ABL, Radiometer, Denmark).

Gas concentrations, fresh gas flow and airway pressures were measured at the proximal end of the endotracheal tube with a standard monitor for respiratory measurements (Capnomac-Ultima®, Datex-Ohmeda, Finland).
Cardiopulmonary data were assessed at three stages: during two-lung ventilation (TLV) before thoracic surgery, during OLV and postoperatively. The measurement of cardiac output was done by thermodilution. The following cardiopulmonary variables were recorded: heart rate (HR), mean arterial pressure (MAP), mean pulmonary arterial pressure (MPAP), central venous pressure (CVP) and pulmonary artery occlusion pressure (PAOP) as well as arterial and mixed venous blood gases. The data were recorded continuously, and systemic vascular resistance (SVR), pulmonary vascular resistance (PVR), oxygen delivery index (DO$_{2i}$), oxygen consumption index (VO$_{2i}$) and shunt fraction were calculated using standard formulas.

Surgical procedures

Open thoracic surgical procedures were performed for established or suspected malignancy (carcinomas, metastases). Patients with infectious processes were excluded. Lung resections were performed through a standard postero-lateral or an antero-lateral muscle-sparing thoracotomy.

Animal experiments

Subjects

The studies were conducted as prospective, randomised, controlled animal experiments. The Animal Ethics Committee of Uppsala University (Sweden) approved the study protocols and the use of animals.

A total of forty-three male and female, 2-3-month-old Yorkshire/Swedish country piglets, weight 27-30kg, obtained from a local breeder, were used. The animals fasted overnight and had free access to water. All pigs underwent the same study protocol (anaesthesia, instrumentation, measurement algorithms, figure 2). The animals were randomly assigned to experimental groups (random numbers generated by EXCEL®, Microsoft Corp., USA).

**Study III and IV:** The animals were randomly divided into spontaneously breathing pigs (SB), pigs that underwent conventional TLV (propofol anaesthesia), the OLV-propofol group (OLV, total intravenous anaesthesia with propofol) and the OLV-desflurane group (OLV, balanced anaesthesia with desflurane).
Animal anaesthesia

A similar anaesthesia protocol was used in all animals in line with the laboratory standard. Anaesthesia was induced by an *i.m.* injection of xylazine (2.2mg·kg⁻¹, Bayer, Germany), tiletamine/zolazepam (6mg·kg⁻¹, Virbac, France) and atropine (0.04mg·kg⁻¹, NM Pharma, Sweden) in all pigs. The SB animals were killed after induction of anaesthesia and bronchoalveolar lavage of both lungs to obtain reference values for normal alveolar and systemic cytokine concentrations in pigs.

Anaesthesia was maintained in TLV pigs by TIVA with fentanyl (Janssen-Cilag, Sweden; 0.38±0.03μg·kg⁻¹·h⁻¹), pancuronium bromide (Organon, The Netherlands; 0.25mg·kg⁻¹·h⁻¹) and propofol (Astra, Sweden; 6.3±0.4mg·kg⁻¹·h⁻¹). In OLV-TIVA pigs (n=6), anaesthesia was maintained by propofol infusion (6.1±0.5mg·kg⁻¹·h⁻¹). The OLV pigs in the desflurane group (n=6) received 6.2±0.2Vol% desflurane (~1 MAC). Anaesthesia in OLV groups was completed by fentanyl (0.35μg·kg⁻¹·h⁻¹) and pancuronium (0.25mg·kg⁻¹·h⁻¹).

The animals received 11±2ml·kg⁻¹·h⁻¹ of isotonic saline during the study. All pigs were killed through an intravenous bolus injection of potassium chloride (150meq) at the end of the study.

Instrumentation

The pigs were instrumented with a left carotid arterial catheter (20G; Becton-Dickinson Critical Care Systems, Singapore), a flow-directed Swan-Ganz thermodilution pulmonary artery catheter (PAC, 7.0French, Baxter, USA), a central venous catheter (4.0French, Becton-Dickinson) and a suprapubic urinary catheter (Sympakath®; Ruesch AG, Switzerland). For volumetric measurements, a 4 French thermistor-tipped femoral arterial catheter (Pulsiocath 4F, Pulsion Medical Systems, Germany) connected to PiCCOplus™ (software version 5.2.2; Pulsion Medical Systems) was inserted into the pigs of study I.

Body temperature was monitored and kept constant by thermoconvection. Pigs were allowed to stabilise for 30min after instrumentation.

Ventilation and haemodynamic measurements

Ventilation and haemodynamic variables [cardiac output, heart rate (HR), mean arterial pressure (MAP), mean pulmonary artery pressure (MPAP), central venous pressure (CVP), pulmonary artery occlusion pressure (PAOP), arterial and mixed venous blood gases] were recorded during baseline TLV, during OLV/TLV in controls, and during TLV after OLV.
The PiCCOplus™ system displayed cardiac index (CI), global end-diastolic volume (GEDV), intrathoracic blood volume (ITBV) and extravascular lung water after injection of 15ml ice-cold normal saline through the central venous catheter.

Systemic vascular resistance (SVR), pulmonary vascular resistance (PVR), oxygen delivery index (DO$_2$i), oxygen consumption index (VO$_2$i) and pulmonary shunt fraction (i.e. venous admixture) were calculated using standard formulas.

**Ventilation management**

After endotracheal intubation (ID 7.0mm; Mallinckrodt, Ireland), the pigs were mechanically ventilated (intermittent positive pressure ventilation, IPPV) with FiO$_2$=0.40 and PEEP=5cmH$_2$O provided by an anaesthesia ventilator connected to a circle system (Maquet KION, Sweden). The orotracheal tube was replaced by an ID 8.5mm tube (Mallinckrodt) after median tracheotomy. An endobronchial blocker (9.0French Arndt Endobronchial Blocker Set, COOK®, Denmark) was bronchoscopically inserted thereafter and was fixed in the left main bronchus. Gas flow, airway pressures and desflurane concentrations were measured at the proximal end of the endotracheal tube with a standard monitor (SC 9000 XL, Siemens, Germany).

The tidal volume was set to 10ml·kg$^{-1}$ in all mechanically ventilated pigs and was not changed throughout the study. Respiratory rate was adjusted to maintain arterial pCO$_2$ of ~40mmHg.

Before and after bronchoscopic manipulation, the lungs of all pigs were recruited by vital capacity manoeuvres using airway pressures of 30-40cmH$_2$O applied to both lungs for approximately 10 seconds.

**OLV and thoracic surgery**

The pigs were put in the right lateral position. The bronchial blocker was inflated under bronchoscopic control. Thus, the ventilation of the left lung was discontinued. Ventilation settings remained unchanged and the tidal volume of 10ml·kg$^{-1}$ was delivered to the dependent lung during OLV.

A left-sided lateral thoracotomy was performed. The thoracic cavity was opened cranial of the diaphragm. The collapse of the left lung was verified by inspection and a surgical procedure was simulated by intermittent manipulation (milking, squeezing) of the collapsed lung for 3min in intervals of 5min.
The bronchial blocker was removed after OLV and the nondependent lung was reinflated by a vital capacity manoeuvre. Afterwards, TLV was re-established and the thoracic incision was closed.

Sample acquisition and processing

Bronchoalveolar lavage

**Study I and II:** The BAL procedure applied follows a standardised method. Before surgery, the correct position of the DLT was confirmed bronchoscopically (Olympus models BF-P40 and BF3-C40), and the first bronchoalveolar lavage (BAL) was done ~30 min after intubation.

The BAL of the dependent ventilated lung was performed by passing the fibre-optic bronchoscope through the endobronchial tube and wedging the tip into a segmental bronchus of the left-sided lower lobe, the right lower or middle lobe. Different randomly chosen segments were subjected to BAL each time. BAL was performed by sequentially instilling normal saline (10-20 ml portions), and gentle aspiration on three occasions: after intubation, immediately after thoracic surgery and 2 hours postoperatively. The returned volume of lavage fluid was not different between the groups.

**Study III and IV:** Following intubation of pigs, bronchoscopy and the first bronchoalveolar lavage (EF-B14/L, Xion Ltd., Germany) were conducted. The BAL was performed in a randomly chosen segmental bronchus of the left and right lower lobes. For BAL, 40 ml of normal saline (Fresenius Kabi AB; Norway) were sequentially instilled in 10 ml portions and suctioned; ~50% of fluid was recovered. The second BAL was done immediately after OLV. After 90 min of postoperative TLV, a third BAL was performed. The recovery rates of lavage fluid did not differ between groups or time intervals.

Preparation of BAL fluid and cell counting

**Study I – IV:** The bronchoalveolar lavage fluid (BALF) was collected in sterile tubes and immediately put on crushed ice. BALF was filtered through sterile gauze filters, collected on ice in siliconised containers and centrifuged (200-250 g, 10 min, 4°C). Supernatant aliquots of 500 μl were snap frozen and kept at -80°C for subsequent analysis. The cell pellets were resuspended in ice-cold phosphate buffer with 0.01% sodium azide and 2% bovine serum for staining and counting (Neubauer haemocytometer). The differentiation of BAL leukocyte subpopulations was performed by flow cytometry (FACScan®, Becton-Dickinson, Germany) in study I and II (figure 3).
Figure 3. Differentiation of alveolar macrophages (R1), lymphocytes (R2) and granulocytes (R3) by their light forward and side scatter properties using FACScan®.

Lung tissue sampling

**Study IV:** The lungs were excised *en bloc* via median sternotomy immediately after the pigs were killed. Tissue samples of approximately 0.125cm³ were harvested from both lungs largest diameter at six representative locations from peripheral (subpleural) to central (parahilar) regions (figure 4). Three samples from each location were obtained, shock frozen in liquid nitrogen and stored at -80°C until analysis.

Figure 4. The origin of representative lung tissue samples for mRNA isolation indicated by black dots, successively obtained from subpleural to parahilar regions of both lungs (study IV).
Measurement of protein and cytokine concentrations

**Study I and II:** The concentrations of interleukin (IL) 8, IL10 and of soluble intercellular adhesion molecule (sICAM)-1 in the BAL fluids were determined by commercially available, quantitative sandwich enzyme immunoassays, (Quantikine®, R&D Systems Ltd., Germany). Tumour necrosis factor (TNF) α and polymorph-nuclear (PMN) cell elastase immunoassays were provided by Immunotech, France and Milenia Biotech, Germany, respectively. Protein concentrations were measured by an assay for the colorimetric detection and quantitation of total protein (Micro BCA™ Protein Assay Reagent Kit, Pierce, USA).

All samples of a single patient were analysed in the same assay run. The samples were measured in duplicates. The assays were conducted in accordance with the manufacturer’s instructions. The optical density of the samples was determined by a microplate reader (Safire®, Tecan Ltd., Austria) and analysed by interpolation from standard curves using the Safire microplate reader software.

Albumin concentrations were estimated by nephelometry (Nephelometer BN 2000, Dade/Behring, Germany)

**Study III:** The concentrations of IL8, IL10 and of TNFα in BAL supernatants and serum samples were determined by quantitative sandwich enzyme immunoassays (Biosource Ltd., Germany; R&D Systems Ltd., Germany). Protein concentrations were measured using the Micro BCA™ Protein Assay Reagent Kit.

All samples of a single pig were analysed in triplicates in the same assay run. The assays were used following the manufacturer’s instructions by an investigator blinded to randomisation and the study protocol. Cytokine concentrations were calculated by interpolation from standard curves on the basis of the data obtained from the Safire microplate reader using SigmaPlot® V. 11 (Systat Software, Inc, USA).

**Analysis of cytokine-mRNA**

**Study IV:** Semiquantitative analysis of IL1β-, IL8-, IL10-, TNFα- and β-actin-mRNA expression was done using reverse transcriptase polymerase chain reaction (RT-PCR). Total RNA was extracted from porcine lung lavage cells and lung tissue samples with TRIZOL®-Reagent (Invitrogen, Germany), following the manufacturer’s protocol, after homogenisation of the cells and tissue samples. Isolated RNA was reversely transcribed into cDNA for 60min at 42°C, using Oligo (dT)_{12-18} primers and RevertAid™ Reverse Transcriptase (Fermentas, Germany). The cDNA (0.5μg) was amplified in a 50μl-reaction containing 0.4μl Taq-Polymerase, 1μl dNTP, 1.5μl MgCl₂, 5μl 10x PCR-buffer (all Invitrogen), and 0.5μl each of sense
and anti-sense primers (MWG biotech, Germany, table 1). The reaction mixture was amplified (denaturation for 30s at 95°C, annealing for 30s, extension for 45s at 72°C) in a Mastercycler® gradient (Eppendorf, Germany).

Table 1. Primer sequences, annealing temperature and the number of cycles for PCR.

<table>
<thead>
<tr>
<th>Target</th>
<th>Sequence (5’- 3’)</th>
<th>Annealing</th>
<th>No. of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1 sense</td>
<td>GAC ACA TGG GAT AAC GAG GC</td>
<td>58°C</td>
<td>33</td>
</tr>
<tr>
<td>IL1 antisense</td>
<td>ACG CAG GAC AGG TAC AGA TT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL8 sense</td>
<td>AGC CCG TGT CAA CAT GAC TTC C</td>
<td>61.3°C</td>
<td>38</td>
</tr>
<tr>
<td>IL8 antisense</td>
<td>GAA GTT GTG TTG GCA TCT TTA C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL10 sense</td>
<td>GCA TCC ACT TCC CAA CAA</td>
<td>60°C</td>
<td>10+28</td>
</tr>
<tr>
<td>IL10 antisense</td>
<td>CTT CCT CAT CTT CAT CGT CAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFα sense</td>
<td>ATC GGC CCC CAG AAG GAA GAG</td>
<td>58.3°C</td>
<td>30</td>
</tr>
<tr>
<td>TNFα antisense</td>
<td>GAT GGC AGA GAG GAG GTT GAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-actin sense</td>
<td>GGA CTT CGA GCA GGA GAT GG</td>
<td>65°C</td>
<td>27</td>
</tr>
<tr>
<td>β-actin antisense</td>
<td>GCA CCG TGT TGG CGT AGA GG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A = adenine, C = cytosine, G = guanine, T = thymine

In case of IL10, a touchdown PCR was performed with the annealing temperature lowered from 65°C to 60°C during the first 10 cycles. All PCR reactions were performed at least twice. The samples of a single pig were analysed in duplicates in the same assay run by one blinded investigator.

Quantification of PCR products

PCR products were analysed by electrophoresis using 2% agarose gels containing ethidium bromide alongside RNA molecular weight markers (100bp, Roche Applied Science, Germany, figure 5). The concentration of the RT-PCR product was normalised to the amount of β-actin-mRNA, which was used as a standard for each sample. The calculations were performed

Figure 5. Representative results of agarose gel electrophoresis of β-actin mRNA and of cytokine-specific mRNA expression for IL8, TNFα, and IL10. The total RNA was extracted from homogenates of representative dependent lung tissue samples of TLV and OLV pigs. bp=molecular weight marker, 0=no-cDNA control, I=pig after TLV, II=pig after OLV with propofol anaesthesia, III=OLV pig undergoing desflurane administration.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, v. 16) and Sigmaplot, v. 11 (SPSS Inc., Chicago, IL, USA). A p value of less than 0.05 was considered significant for all statistical procedures.

Study I: The hypothesis was tested that after OLV cell numbers, IL8 or PMN elastase would increase by more than 25% with a power of 0.8 and a two-tailed p of less than 0.05. This required a sample size of 12 to 14 subjects in each group.

Study II: Power calculations were based on previous data. By estimation, 15 patients per group were needed to detect differences in alveolar cytokine concentrations and alveolar leukocyte count between propofol and desflurane anaesthesia using a two-sided design at a significance level of 5% (α=0.05) with a probability of 80% (β=0.20).

Study III and IV: Power calculation revealed that at least 5 animals per group were needed to detect differences in alveolar IL8 and TNFα-concentrations between controls and OLV pigs using a two-sided design at a significance level of 5% (α=0.05) with a probability of 80% (β=0.20).
Furthermore, previously published data of pigs that underwent a similar experimental setup [8-9] revealed a minimum of 4 animals per group to detect differences in DAD scores and in the extent of neutrophil infiltration between OLV pigs and controls.

The data were tested for normal distribution with the Shapiro-Wilks W test. The data are presented as mean±standard deviation in the case of normal distribution (cardiopulmonary, gas exchange and ventilation variables) and are expressed as median and interquartile range (P_{25}-P_{75}) in the case of non-normal distribution.

The analysis of normally distributed data was performed by a repeated measures one-way analysis of variance with post-hoc Bonferroni correction. Non-normally distributed data were logarithmically transformed to achieve homogenous variances of data sets; however, cytokine concentrations in BAL fluids still differed from normal distribution even after transformation.

In study I and IV, the data were analysed using Kruskal-Wallis H-Test with adjustment of α-level for repeated measurements. In study II and III, sequential changes of the variables with the co-factor time in each group were confirmed by a repeated measures general linear model (type III sums of squares). The differences between the two groups before and after OLV were assessed by calculation of the differences in each parameter and evaluation by linear regression analysis.

In some cases, mediator concentrations were below the detection limits of the assays. These values were included into the statistical analysis with a value of 0.01.
RESULTS

Patient characteristics in study I and II

Biometric data were equally distributed among the groups that underwent open thoracic surgery. Preoperative lung function tests (FVC, FEV₁, \( \text{paO}_2 \), \( \text{paCO}_2 \)) revealed normal values in all patients. The surgical admission criteria included patients scheduled for lobectomy, atypical pulmonary resection and pneumonectomy.

The duration of one-lung ventilation in relation to the time of surgery was not different between the groups. The co-factors ASA status, age, gender, smoking history and left- or right-sided thoracotomy did not influence the time course of immune parameters as analysed by linear regression together with the factors duration of OLV and duration of surgery.

There were also no differences in the use of blood products during and after surgery and no correlation between immune parameters and the administration of erythrocytes.

All patients had an uneventful postoperative course. There were no differences in time to extubation, postoperative morbidity and in length of hospital stay between the patients.

The effects of OLV on haemodynamics, ventilation and gas exchange

Studies I and II: The delivery of the entire tidal volume to the dependent lung increased peak and mean airway pressures during OLV. The OLV-induced rise in mean pulmonary artery pressure (MPAP), pulmonary arterial occlusion pressure (PAOP), central venous pressure (CVP) and intrapulmonary shunt (i.e. venous admixture, \( Q_s/Q_t \)) were observed in all patients without differences between the groups. The calculated transmural pulmonal-capillary pressures, estimated as being halfway between MPAP and left atrial pressure (i.e. PAOP) [40] increased up to 16-18mmHg during OLV in the patients. Mean arterial pressure (MAP), heart rate (HR) and cardiac index (CI) remained unchanged.

The mean postoperative haemodynamic and ventilation data normalised to preoperative values in all patients.
**Study I:** OLV with low or high $V_T$ resulted in different peak and plateau airway pressures ($P_{AW}$ peak, $P_{AW}$ plateau) but not in $paO_2$ differences during OLV. However, calculation of effective airway pressures (driving pressure calculated as difference of $P_{AW}$ plateau and PEEP) revealed no differences between both patient groups during OLV (figure 6).

During mechanical ventilation with $V_T=5\text{ml}\cdot\text{kg}^{-1}$, a higher ventilation rate was required to achieve the desired $paCO_2$ range, whereas minute volume ventilation was not different. Nevertheless, $paCO_2$ was higher in patients who received OLV with $V_T=5\text{ml}\cdot\text{kg}^{-1}$, which represents increased dead space ventilation.

**Study II:** There was also no effect of propofol or desflurane administration on cardiopulmonary variables and gas exchange.

*Figure 6.* Peak and effective airway pressures in patients undergoing OLV either with $V_T=10\text{ml}\cdot\text{kg}^{-1}$ or $V_T=5\text{ml}\cdot\text{kg}^{-1}$ (mean+SD) before OLV and surgery ($t_1$), after 20min of OLV ($t_2$) and at the end of the surgical procedure ($t_3$). The symbol * indicates differences between both groups ($p<0.05$).
Studies III and IV: In TLV pigs and in OLV pigs before intervention, haemodynamic, ventilation and gas exchange data reflected typical values of mechanically ventilated pigs.

The initiation of OLV increased MPAP, PAOP and intrapulmonary shunt (Qs/Qt). Likewise, paO₂ was reduced in OLV pigs but remained constant in animals with TLV. OLV put up peak and mean airway pressures in comparison with the data before, after OLV, and with the data of the TLV group. The administration of either propofol or desflurane did not influence cardiopulmonary variables.

During the period of TLV after OLV, cardiopulmonary and ventilation data returned to the initial values without differences between OLV pigs that received propofol or desflurane. Haemodynamics and gas exchange remained constant in TLV pigs throughout the study.

The volumetric measurements (EVLW, GEDV and ITBV) revealed no differences between TLV and OLV pigs. These variables remained unchanged in all pigs during study III.

The alveolar immune effects of different tidal volumes during OLV (study I)

Intra-alveolar cell numbers and protein concentrations

In both high and low Vₜ groups, the number of intra-alveolar cells, protein and albumin concentrations increased during the observation (table 2). In patients ventilated with Vₜ=5ml·kg⁻¹, the increase of cells, protein and albumin concentrations was significant in the BAL fluid after OLV and in the postoperative course.

The relative fractions of granulocytes, lymphocytes and alveolar macrophages changed in the BAL over time. The subpopulations were differentially distributed in both groups. FACS analysis of BAL cells revealed a decreased number of granulocytes and an increased fraction of alveolar macrophages in patients ventilated with lower Vₜ.
Table 2. Differentiation of BAL cell subpopulations in study I (per cent of the total cell numbers). The data are displayed as medians and interquartile ranges. The symbols * and # indicate significant differences within each group, § indicates differences between both groups (p<0.05).

<table>
<thead>
<tr>
<th>Time</th>
<th>TLV, preoperatively</th>
<th>after OLV</th>
<th>TLV, postop.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$V_T$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10ml/kg</td>
<td>5ml/kg</td>
<td>10ml/kg</td>
</tr>
<tr>
<td>BAL cells</td>
<td>[10^5·ml^{-1}]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>[1.1]</td>
<td>[0.4]</td>
<td>[1.4]</td>
</tr>
</tbody>
</table>

Pro-inflammatory mediators

In all patients, alveolar concentrations of PMN elastase, IL8 and TNFα increased during and after OLV. In contrast to patients ventilated with $V_T=10ml·kg^{-1}$, mechanical ventilation with lower $V_T$ resulted in reduced alveolar TNFα concentrations immediately after OLV (figure 7).

Adhesion molecule sICAM-1

The concentrations of sICAM-1 were significantly decreased in the BAL of patients ventilated with $V_T=5ml·kg^{-1}$ in comparison with patients in the high $V_T$ group (figure 7).

Anti-inflammatory IL10

Intra-alveolar IL10 concentrations decreased during ventilation with $V_T=10ml·kg^{-1}$ but remained unchanged in the low $V_T$ group.

The time courses of intra-alveolar cell numbers, protein and albumin concentrations as well as of IL8, PMN elastase and IL10 with exception of TNFα did not differ between the groups after OLV and 2h postoperatively. Intra-alveolar sICAM-1 concentrations were decreased during ventilation with $V_T=5ml·kg^{-1}$.

Thirty minutes of two-lung ventilation with $V_T=5ml·kg^{-1}$ in contrast to $V_T=10ml·kg^{-1}$ resulted in decreased alveolar concentrations of protein, albumin, sICAM-1 and IL10 before thoracic surgery.
Figure 7. The time course of the alveolar pro-inflammatory markers TNFα and sICAM-1 before (I), immediately after OLV (II) and 2 hours postoperatively (III). The mediator concentrations in the BAL fluid are presented as ranges, medians and interquartile ranges, P_{25}-P_{75}. The symbols * and # indicate significant differences within each group, § marks differences between both groups (p<0.05).

The alveolar immune effects of anaesthetic drugs (study II)

In general, the total number of intra-alveolar cells and pro-inflammatory mediators increased in the BAL fluids of patients in study II during and after OLV, which confirms the results of study I (table 3). The mean doses of anaesthetics used in this study were 4.1±1.4mg·kg^{-1}·h^{-1} propofol and 5.6±0.4 volume per cent desflurane.

Intra-alveolar cell numbers and protein concentrations

In the propofol group, the number of intra-alveolar cells increased over time. In contrast, the cell numbers in the desflurane group did not change over time, from TLV before OLV to postoperative TLV. Hence, postoperative BAL cell numbers were higher in the propofol group than in the desflurane group (table 3).

The differentiation of BAL cells using their light scatter properties revealed that the proportion of alveolar granulocytes and lymphocytes differed between both groups, whereas the fraction of alveolar macrophages remained constant. Postoperatively, the number of granulocytes was
increased and the lymphocyte fraction was reduced in the propofol group (figure 8).

**Table 3.** Total number of cells in the BAL fluids of study II. The data are presented as medians [interquartile range] in each group. * indicates differences within the propofol group, § denotes differences between both groups (p<0.05).

<table>
<thead>
<tr>
<th>Time</th>
<th>preoperative TLV</th>
<th>after OLV</th>
<th>postoperative TLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>propofol</td>
<td>desflurane</td>
<td>propofol</td>
</tr>
<tr>
<td>BAL cells [10⁶·ml⁻¹]</td>
<td>0.6 [0.6]</td>
<td>0.6 [1.1]</td>
<td>0.8 [1.0]</td>
</tr>
<tr>
<td></td>
<td>1.7[2.0]*</td>
<td>1.0[0.7]§</td>
<td></td>
</tr>
</tbody>
</table>

*Figure 8.* The relative numbers of granulocytes and alveolar macrophages in the BAL fluids of study II patients before (I), after OLV (II) and postoperatively (III). The data are presented as medians and interquartile ranges (P25-P75). The symbol * indicates difference within the propofol group, § indicates differences between both groups, p<0.05).

The median BAL protein and albumin concentrations increased in both groups over time. However, the total protein concentrations were significantly lower in the desflurane group during preoperative TLV.
Pro-inflammatory mediators

The alveolar concentrations of PMN elastase, IL8 and TNFα increased in all patients during and after OLV. The pro-inflammatory effects of OLV were attenuated in the desflurane group. Likewise, the increases of alveolar PMN elastase and IL8 were to a lesser extent pronounced in these patients (figure 9).

Adhesion molecule sICAM-1

Alveolar sICAM-1 did not change during and after OLV, whereas desflurane anaesthesia decreased sICAM-1 concentrations before and after OLV as well as 2 hours postoperatively.

Alveolar IL10 expression

Intra-alveolar IL10 concentrations were decreased in the propofol group but remained unchanged during and after desflurane administration. The release of IL10 into the alveoli was reduced in patients who received desflurane.

Figure 9. Time-dependent changes of the alveolar inflammatory markers IL8 and PMN-elastase in the BAL fluids of study II before (I), after OLV (II) and 2h postoperatively (III). The data are presented as ranges, medians and interquartile ranges (P25-P75); the symbol * indicates differences within propofol group, # marks changes within the desflurane group, § indicates differences between both groups, p<0.05.
The pulmonary immune effects of OLV, thoracic surgery and different anaesthetics in a porcine model (study III)

Animals with spontaneous breathing and two-lung ventilation

Conventional TLV in pigs did not increase the number of BAL cells and alveolar protein concentrations. TLV had also no effects on the release of alveolar pro-inflammatory cytokines (IL8 and TNFα). The time-dependent cytokine changes in the BAL fluid of TLV pigs and in the peripheral blood were insignificant, and they were not different to mediator concentrations in spontaneously breathing animals.

BAL cell numbers and protein concentrations in OLV pigs

The number of intra-alveolar cells increased in all OLV pigs during the study period (figure 10, 11). In OLV-propofol pigs, there were no differences between the ventilated and non-ventilated lungs. However, the alveolar cell numbers in the nondependent collapsed lungs of OLV-desflurane pigs were stronger pronounced by lung collapse and surgery (figure 11). The total protein concentration in the lavage fluids was not influenced by OLV and surgery.

Figure 10. The time-dependent changes of intra-alveolar cell numbers in the BAL fluid in spontaneously breathing controls (SB), in TLV pigs (baseline, 90min, 180min of TLV) and in animals undergoing OLV (TLV before OLV, after OLV and 90min thereafter), in the dependent, ventilated lung. The data are presented as ranges, medians and interquartile ranges (P25-P75). * denotes differences within the propofol OLV group, # indicates changes within the desflurane OLV group and $ marks differences between OLV groups and animals with TLV (p<0.05).
Figure 11. The time course of BAL cells in SB pigs, during TLV (baseline, 90min, 180min of TLV) and in animals undergoing OLV in the nondependent lung (medians and interquartile ranges (P25-P75). * marks differences within the propofol OLV group, # indicates changes within the desflurane OLV group, § denotes differences between both OLV groups, and $ indicates differences between OLV groups and animals with TLV, p<0.05.

Pro-inflammatory IL8 and TNFα in OLV pigs

In all OLV-pigs, the alveolar concentrations of pro-inflammatory cytokines increased during OLV and surgical manipulation. TNFα concentrations were not different between both OLV groups but were enhanced in the non-dependent, surgically manipulated lungs (figure 13). The time course of IL8 was influenced by general anaesthesia in the ventilated lungs. The concentration of IL8 in the BAL was reduced by desflurane inhalation during and after OLV (figure 12).

Anti-inflammatory IL10

The alveolar concentrations of IL10 were in the range of the detection limits of the used cytokine assay in all animals after the defined time intervals.

Systemic expression of cytokines induced by OLV and lung manipulation

The serum IL10 concentrations were significantly lower in OLV pigs that received desflurane and remained decreased throughout the study period.
Likewise, desflurane suppressed the serum concentrations of IL8 and TNFα in these animals during and after OLV (figure 14).

Figure 12. The time course of IL8 expression in BAL fluids of both the dependent and nondependent lungs of SB pigs, of TLV pigs (baseline, 90min, 180min of TLV) and of animals undergoing OLV (TLV before OLV, after OLV and 90min thereafter). The data are presented as ranges, medians and interquartile ranges (P_{25}-P_{75}). The symbol * indicates differences within the propofol group, # within the desflurane group, § marks differences between both OLV groups and $ differences between OLV pigs and animals with TLV (p<0.05).
Figure 13. The course of alveolar TNFα concentrations in the dependent and nondependent lungs of SB pigs, of TLV pigs (baseline, 90min, 180min of TLV) and of animals undergoing OLV (TLV before OLV, after OLV and 90min thereafter). The data are displayed as medians and IQR (P_{25}-P_{75}). The symbol * denotes differences within the propofol group, # within the desflurane group, § indicates differences between both OLV groups and $ differences between OLV pigs and animals with TLV (p<0.05).
Figure 14. Changes of serum IL8 and TNFα concentrations in the different study groups. The data are displayed as ranges, medians and IQR (P25-P75); the symbol * indicates differences within OLV-propofol pigs, # changes within the animals receiving desflurane, § indicates differences between both OLV groups, and $ differences between OLV pigs and animals with TLV (p<0.05).
Expression of cytokine-mRNA in BALF cells (study IV)

In contrast to the total alveolar cell numbers in the BAL of TLV pigs, which remained constant throughout the study period, the number of cells increased in the BAL of both the OLV-propofol and OLV-desflurane group over time (table 4). The number of cells in BAL increased also following lung collapse and surgical manipulation of the nondependent lungs.

The alveolar expression of pro-inflammatory IL8-mRNA followed the recruitment of cells into the alveoli of OLV pigs without any differences between the ventilated and temporarily collapsed lungs (figure 15). The IL8-mRNA concentration in the BAL cells was also not different between pigs that received propofol or desflurane anaesthesia.

The concentration of anti-inflammatory IL10-mRNA in BAL cells was not affected by OLV, surgical manipulation and administration of different anaesthetics. Correspondingly, the expression of IL1β- and TNFα-mRNA by BAL cells remained unchanged during the experiment.

Table 4. The increase of cells in the BAL fluids [x1000·ml⁻¹] of pigs undergoing OLV and thoracic surgery with either propofol or desflurane anaesthesia is presented separately for the ventilated and temporarily collapsed lungs as medians [IQR]. The symbol * marks differences within the propofol OLV group, # indicates changes within the desflurane OLV group, (p<0.05).

<table>
<thead>
<tr>
<th></th>
<th>TLV before OLV</th>
<th>after OLV</th>
<th>90min TLV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>propofol</td>
<td>desflurane</td>
<td>propofol</td>
</tr>
<tr>
<td>Ventilated</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 15. Time-dependent changes of mRNA expression of pro-inflammatory IL8 in the BAL cells of pigs undergoing TLV or OLV and thoracic surgery. The data are grouped by origin (dependent, nondependent lung) and by the type of anaesthesia. The symbol * indicates differences within the propofol OLV group, # indicates changes within the desflurane OLV group, and $ indicates differences between OLV groups and animals with TLV, p<0.05.

Expression of cytokine-mRNA in lung tissue
Neither mechanical TLV nor OLV and temporary lung collapse with subsequent simulation of a surgical procedure affected the lung tissue expression of pro-inflammatory IL1β, IL8, TNFα and anti-inflammatory IL10-mRNA in relation to the expression of β-actin mRNA in the animals.
The expression of mRNA was equally distributed and there were also no differences between subpleural, intermediate and parahilar lung tissue samples of the left and the right lungs (figure 16). The mRNA concentrations were also not different between lung tissue samples of pigs that received general anaesthesia with propofol or desflurane.

Figure 16. The mRNA-expression of pro-inflammatory IL8, TNFα, IL1β and anti-inflammatory IL10 in relation to β-actin mRNA in the lung tissue of TLV and OLV pigs. The data are presented separately for the non-dependent and dependent lungs as ranges, medians and interquartile ranges (P25-P75). The dots indicate the outliers in each group.
DISCUSSION

Summary of main results

**Study I:** The data indicate that OLV increased the release of pro-inflammatory mediators in the dependent, ventilated lung. The reduction of tidal volume and the subsequently decreased peak airway pressure have significant effects on alveolar TNFα, sICAM-1 and IL10 concentrations. The suppression of IL10 in the high V_T group suggests an improved anti-inflammatory immunoregulation in the low V_T group. The differences in alveolar sICAM-1 concentrations affirm the supposition that protective ventilation is able to protect alveolar cells from activation during OLV.

**Study II:** The administration of the volatile anaesthetic desflurane modulates the alveolar immune response induced by OLV as indicated by decreased expression of pro- and anti-inflammatory alveolar mediators. Propofol anaesthesia in contrast resulted in higher numbers of alveolar cells and a relative increase of the alveolar granulocyte fraction associated with decreased quantity of alveolar lymphocytes. The cytokine pattern followed the different recruitment of granulocytes into the alveoli during propofol and desflurane anaesthesia since PMN cells are the main source of IL8 and elastase.

**Study III:** The experimental data confirm that OLV induces a pro-inflammatory response in the alveoli of the dependent, ventilated lung. It is characterised by time-dependent recruitment of alveolar cells and by increased IL8 and TNFα concentrations in the BAL fluid of pigs. Temporary lung collapse and surgical manipulation result in an immune response of nearly the same extent in the nondependent lung. Likewise, IL8 and TNFα concentrations in the peripheral blood were higher in OLV-propofol pigs but not in animals that underwent standard TLV. Anaesthetic drugs modulate the pro-inflammatory response in pigs, indicated by decreased alveolar and serum concentrations of IL8 and TNFα following desflurane administration. Related to the characteristics of a volatile anaesthetic, the alveolar IL8 expression in the non-ventilated, temporarily collapsed lung was not attenuated by desflurane.

**Study IV:** OLV, temporary lung collapse and surgical manipulation increased the expression of IL8-mRNA in alveolar cells in a time-dependent manner. The administration of different anaesthetic drugs did not modulate
the mRNA expression in OLV pigs indicated by unaffected cytokine mRNA concentrations following desflurane or propofol anaesthesia.

**OLV-induced lung injury**

Different forces applied to the lung tissue such as mechanical stress [27], cyclic tidal recruitment of alveoli [41], lung collapse and atelectasis associated with alveolar hypoxia [42] and reperfusion-induced formation of oxygen and nitrous radicals [15] induce a complex network of defence mechanisms. The upward regulation of alveolar cytokines [41] and the recruitment and activation of cells play a major role in the pulmonary response to stress [43-44]. The direct trauma to the pulmonary blood-gas barrier by ventilation-induced stress [19], by direct manipulation or by the loss of endothelial and alveolar epithelial cell integrity [45] results in the release of intracellular cytokines to the interstitium and decompartmentalisation into both the alveoli and systemic circulation [46]. Hence, the inflammatory reaction is not limited solely to the lungs but also includes a systemic immune response that may affect distal organs [15]. Therefore, in ARDS patients, not the respiratory failure, but more frequently the multiple organ dysfunction is the primary cause of death [47-48].

The clinical significance of pulmonary inflammatory reactions to thoracic surgery and OLV was demonstrated in patients undergoing surgery for lung cancer. The concentrations of leukotriene B4, hydrogen peroxide and hydrogen ions in exhaled breath condensates increased significantly after lobectomy, indicating inflammation and oxidative stress [49]. However, analysis of exhaled biomarkers does not allow the differentiation of immune effects between the ventilated and non-ventilated lung.

Mechanical ventilation with relatively high tidal volumes and subsequently increased peak airway pressures results in alveolar damage [8-9] and may promote ventilation-induced lung injury [23, 27, 29]. Additional forces result from increased transmural pulmonary capillary pressure [50] during OLV, enhanced by lateral positioning. Pulmonary capillary pressures [40] were most likely increased in the dependent lung as indicated by increased MPAP, PAOP, and CVP in patients during OLV. High pulmonary capillary pressures were accompanied by disruption of the alveolo-capillary barrier in recent experiments [45, 50-51]. In patients, at mid-lung level, the capillary pressure is about 16mmHg. However, the capillaries of the dependent lung in lateral decubitus position are localised 15-20cm lower. This results in an additional hydrostatic gradient and alters the distribution of pulmonary perfusion [8]. In fact, OLV and surgical manipulation induce an important ventilation/perfusion mismatch secondary to hyperperfusion of the ventilated lung and hypoperfusion of the manipulated lung. The
hyperperfusion in the dependent lung persisted postoperatively, despite normalisation of haemodynamic and ventilation variables after OLV [8].

The increased albumin concentrations in the BAL fluids of patients that underwent OLV may thus be related to the insufficiency of the pulmonary blood-gas-barrier induced by increased pulmonary capillary pressures. It is notable that MPAP and PAOP were increased to the same degree in all patients during OLV. This may explain why alveolar albumin concentrations did not differ between the groups.

Cytokines in OLV-induced lung injury

In alveolar injury, cytokines transmit signals between the cells that are involved in the inflammatory process [52]. TNFα and IL8 are the most important pro-inflammatory mediators expressed by activation of alveolar macrophages and neutrophil granulocytes in the lung [53]. TNFα is a polypeptide cytokine, in principle produced by macrophages/monocytes. It is commonly associated with critical inflammatory conditions [54]. Previous experimental data suggest that alveolar macrophages produce less IL1 but more TNFα than plasma monocytes [55]. The different behaviour may explain the unchanged TNFα plasma concentrations during conventional mechanical ventilation in anaesthetised patients [56] and during TLV in pigs. TNFα in the BAL fluids therefore reflects the secretion of alveolar macrophages in the present studies. The observation is consistent with previously reported pulmonary histological changes including macrophage aggregation and neutrophil influx [30-31, 57].

Interleukin 8 is one of the central cytokines that are responsible for the recruitment of inflammatory cells into the alveoli. It binds to specific cellular receptors and promotes the adherence and activation of neutrophils [58]. IL8 is therefore increased in the BAL fluid of patients with ARDS, sepsis and multiple organ failure [59], and alveolar IL8 concentrations have been correlated to the incidence of postoperative pulmonary complications after oesophagectomy [60]. The cytokine is a powerful chemotactic factor for polymorphonuclear neutrophils and stimulates the adherence of PMN to pulmonary epithelial and endothelial cells. The alveolar IL8 release and the increased expression of the IL8-mRNA accompany the increased cell numbers in the BAL fluids. After activation, neutrophils liberate potentially toxic mediators into the alveolar space such as PMN elastase, leukotrienes and free oxygen radicals. These substances are considered to be potentially harmful to the host tissue [61].

In addition, alveolar epithelial cells express molecules like ICAM-1, which interact with leukocytes and promote the transepithelial migration of PMN [62]. The expression of sICAM-1 was reduced by OLV with
VT=5ml·kg⁻¹ and by desflurane administration in patients, which suggests that protective ventilation and volatile anaesthetics are able to prevent alveolar cells from activation. Accordingly, the cytokine IL10 diminishes the severity of lung injury. The balance between pro-inflammatory (TNFα, IL-8) and anti-inflammatory cytokines is essential to directing the pulmonary immune response [68]. Anti-inflammatory IL10 is generated in response to cell activation and modulates the extent of cellular activation by reducing the expression of pro-inflammatory cytokines [60].

The suppression of IL10 during ventilation with high VT but not in patients with low VT and in subjects who undergo desflurane anaesthesia indicates an improved anti-inflammatory immune regulation in these groups. In pigs, the alveolar IL10 concentrations were in the range of the detection limit of the used cytokine assay, which indicates that the porcine pulmonary biotrauma was to a lesser extent pronounced in comparison with patients [69]. This is supported by only minor changes in pulmonary haemodynamics, by only a small increase of intrapulmonary shunt and by unchanged volumetric data in pigs. Nevertheless, desflurane suppresses the serum IL10 concentrations in animals, which indicates the presence of a systemic anti-inflammatory immune response.

Effects of anaesthetic drugs in OLV-induced lung injury

The sequestration of activated neutrophils into the lung is an early but prominent signal in the alveolar inflammatory process [44]. Lipid-soluble drugs such as propofol may reduce granulocyte recruitment by reduction of polarisation, chemotaxis and inhibition of the respiratory burst in clinically used concentrations [63], thus explaining the unchanged cell numbers and cytokine expression in TLV pigs. However, the pro-inflammatory response in OLV-propofol pigs should not be interpreted as being increased by propofol administration. This immune reaction was unquestionably diminished as well but to a lesser extent than in pigs that received desflurane. It has to be acknowledged that the present model of thoracic surgery is not able to determine the origin of increased serum cytokine concentrations in the animals.

The number of alveolar cells was not enhanced in the nondependent lungs of OLV pigs related to the inhibition of leukocyte attraction by propofol. The activation of neutrophils in contrast was inhibited by desflurane anaesthesia, indicated by decreased alveolar IL8 concentrations. The result confirms recent data on decreased pro-inflammatory alveolar mediator release to endotoxin-induced lung injury in mice by isoflurane [64], to thoracic surgery in the nonventilated lung by sevoflurane [65] and to OLV by desflurane [II]. Desflurane and propofol administration modulate to a different degree the
recruitment of leukocyte subpopulations into the ventilated lung of patients during OLV. This is linked to a different expression of cytokines in the alveoli [II]. Moreover, volatile and intravenously administered anaesthetic drugs may also affect the activation of immunocompetent cells in the peripheral blood, which contributes to decreased serum cytokine concentrations in OLV pigs. That is of importance since the administration of volatile anaesthetics prevents the organism from systemic pro-inflammatory response and may thus improve the clinical outcome [65].

Recent data on inhibitory effects of halogenated agents on the secretion of pro-inflammatory mediators [66] support the effect of decreased neutrophil activation by desflurane. Whereas in healthy subjects the effects of halogenated agents are transient, experimental results revealed inhibitory effects on neutrophil function [67]. In alveolar epithelial type II cells from rats, halothane, enflurane and isoflurane reduced the secretion of IL6, macrophage inflammatory protein-2 (MIP-2), and monocyte-chemoattractant protein-1 (MCP-1) in a time- and dose-dependent manner, in association with a decrease in MIP-2- and TNFα-mRNA expression [66]. The underlying mechanism was identified as interaction of volatiles with inducible NO synthetase by reversible inhibition of voltage-dependent calcium channels and subsequently decreased intracellular calcium concentrations. In contrast, exposure to volatile anaesthetics and mechanical ventilation increased pro-inflammatory cytokine gene expression in rats [57]. However, inconsistent data of decreased or even improved immune function may be related to different study populations, methodology and laboratory techniques [68].

The unchanged alveolar TNFα concentrations accompanied by decreased expression of IL8 in the ventilated lungs of the OLV-desflurane pigs suggest that desflurane may exert its suppressive effects mainly on the immune pathways of neutrophils by modulation of granulocyte activation [66]. When considering the higher number of cells in the nondependent lungs of OLV-desflurane pigs, the suppressive effect of desflurane on alveolar IL8 becomes even more important.

The alveolar cytokine expression in pigs demonstrates that OLV is as injurious as a period of complete lung collapse and surgical manipulation. The expression of IL8-mRNA was increased accordingly in alveolar cells of the ventilated lung indicating the pro-inflammatory response. Temporary lung collapse and manipulation increased the IL8-mRNA concentration to the same extent in the alveoli of the nondependent lung. However, the administration of different anaesthetic drugs did not change the concentrations of cytokine mRNA in the BAL cells of OLV pigs. Likewise, anaesthetic management did not influence the mRNA expression in lung tissue samples obtained from representative locations of both lungs, indicating an exclusive effect of anaesthetics on the activation of alveolar cells in the present model.
Manipulation-induced alveolar damage may result from direct mechanical stress but also from alveolar hypoxia during temporary lung collapse. \textit{In vitro} studies established that hypoxia enhances the expression of adhesion molecules on alveolar epithelial cells with subsequently increased neutrophil adherence, an important mechanism in hypoxia-induced lung injury [38].

### Relevant mechanisms for alveolar damage after thoracic surgery

Mechanical ventilation results in significant alveolar damage [9] even in previously healthy lungs [18]. However, alveolar injury may be resolved in normal lungs within hours after mechanical ventilation [22]. During OLV, mechanical stress is additionally increased by enhanced cyclic tidal recruitment of alveoli, indicated by lower compliance and increased airway pressures in patients and OLV pigs. This atelectrauma was associated with persistently decreased dependent lung density and increased lung volume in pigs undergoing OLV [33] and is considered to be the main mechanism in the pathogenesis of ventilated lung injury [41]. As a consequence, OLV-induced lung injury definitely affects the postoperative course, illustrated by an increased incidence of postoperative pulmonary complications, which were correlated to the use of high tidal volumes in thoracic surgical patients [69].

The surgical lung damage strongly depends on time and it seems possible that it even exceeds the OLV-induced lung injury after extended surgery. Clinical data support this conjecture. Recent studies have described the incidence of postoperative pulmonary complications as a function of duration, extent and location of thoracic surgery [10-11, 13-14]. However, the re-expansion of a previously collapsed lung is accompanied by a hypoxia/reperfusion-type response, which results in biochemical and functional changes not just in the previously collapsed lung, but also in the contralateral lung and distant organs [15]. It is associated with a considerable rise in systemic markers of oxidative stress that correlates with postoperative outcome [70].

The reventilation/reperfusion injury seems to be of minor importance in the used animals since the second lavage was performed immediately after lung recruitment and reventilation. Concurrently, experimental data demonstrate that lung recruitment is less deleterious than persistent atelectasis [71]. The repeated alveolar recruitment manoeuvres in both OLV and TLV pigs did also not substantially contribute to the pro-inflammatory response since ARM did not induce cytokine release in TLV pigs.
The finding of a truly asymmetric lung injury in the ventilated lung after thoracic surgery [36] was attributed to OLV-induced alveolar damage. However, this is difficult to explain on the basis of the present results. In patients who developed ALI/ARDS after pulmonary resection, the coincidence of OLV-induced lung injury, postoperatively persistent ventilation/perfusion mismatch and hyperperfusion in the ventilated lung after OLV [8] may have contributed to the pathogenesis of ALI and ARDS.

Modern thoracic surgery includes an increasing number of video-assisted thoracoscopic procedures with limited lung manipulation that may lead to a lesser pronounced lung damage [12]. Therefore, lung protective ventilation strategies are needed to reduce potentially harmful OLV-induced pulmonary damage. It remains to be studied whether protective ventilation modes, the use of different PEEP levels or even techniques using spontaneous breathing may result in less severe lung injury and improve outcome.

Limitations of the clinical studies

The major limitation of the clinical studies includes the sample size and the lack of true blinding. It should be noted that outcome variables were not specified and recorded in the clinical studies I and II. The relationship between cytokine profiles and organ damage or infectious complications could also not be clarified. Following the design of study I, the patients were ventilated with $V_T=5\text{ml/kg}^{-1}$ or $10\text{ml/kg}^{-1}$ immediately after tracheal intubation. In study II, the patients had already received propofol or desflurane for 30 min, since the first BAL had to be performed with delay after intubation. Therefore, a control BAL of all patients during two-lung ventilation was not performed to limit the number of BAL procedures, which may affect the pulmonary inflammation by themselves. In fact, it cannot be precluded that the differences before open thoracic surgery are partially influenced by the first BAL. However, the studies I and II reflect clinical routine procedures, and therefore, differences in baseline measurements were unavoidable. Moreover, reliable controls can hardly be obtained in such clinical observations.

Limitations of the experimental studies

The major limitation of the animal experiments is the fixed ventilation setup. Whereas FiO$_2$ was limited to 0.4 in order to avoid direct toxic effects of oxygen [72], the use of $V_T=10\text{ml/kg}^{-1}$ may have overestimated OLV-induced lung injury. Protective lung ventilation strategies with $V_T=5-6\text{ml/kg}^{-1}$ are...
accepted in modern thoracic anaesthesia, since the pro-inflammatory alveolar response is attenuated in patients by protective ventilation during OLV [I]. However, the normal lungs of healthy pigs [73] are different to the incompliant lungs of adult patients. Thus, OLV and surgical manipulation in normal pigs are not as injurious as in patients that undergo thoracic surgery. The unchanged cytokine mRNA levels obtained from lung tissue samples may support this statement.

Otherwise, enhanced cyclic opening and collapse of alveoli, even at low inspiratory pressures and low inspiratory volumes may increase stretch and shearing forces, which result in lung injury and surfactant dysfunction [41, 74]. The alveolar trauma could be attenuated by application of sufficient positive end-expiratory pressure (PEEP), which would even outweigh the concomitant increase in inspiratory pressure [75].

The applied tidal volumes of 10ml·kg\(^{-1}\) were accompanied by respiratory frequencies of >20/min in pigs suggesting that lower V\(_T\) (5-6ml·kg\(^{-1}\)) may result in increased dead space ventilation, which may have influenced the results.

The design of the studies III and IV was closely related to a clinical setting and to our recently published animal model of thoracic surgery [8, 33]. This model is limited to 60min of OLV and 90 minutes follow-up. Longer periods of OLV may increase the damage to the ventilated lung [76]; alternatively the lung injury may be reversed in the case of a longer postoperative period [22].
CONCLUSIONS

The overriding objective of the present studies was to demonstrate the alveolar and systemic immune effects of mechanical ventilation and thoracic surgery in clinical observations and in a porcine model. The main result is that one-lung ventilation results in epithelial injury, leukocyte recruitment and expression of pro-inflammatory mediators in the alveoli of the ventilated lung. A pro-inflammatory response was also detected in the surgically manipulated lung, which suggests that both OLV and a period of lung collapse and manipulation may be injurious to a similar degree.

These pro-inflammatory responses may contribute to increased postoperative pulmonary complications in patients scheduled for thoracic surgery.

The extent of the alveolar immune response was decreased by protective mechanical ventilation using lower tidal volumes. The volatile anesthetic desflurane decreased the alveolar cytokine release by modulation of alveolar leukocyte recruitment and activation. Likewise, systemic immune responses induced by OLV and thoracic surgery were attenuated by the type of general anaesthesia.

The results of these studies could help to implement lung protective ventilation approaches in thoracic surgery and may therefore contribute to improved patient care and outcome.

(1) One-lung ventilation during thoracic surgery induces significant alveolar injury and increases the expression of pro-inflammatory mediators in the alveoli of the dependent lung in a time-dependent manner.

(2) A protective ventilation approach that is based on lower tidal volumes has significant effects on pro-inflammatory alveolar TNFα concentrations during and after one-lung ventilation. The suppression of anti-inflammatory IL10 and increased pro-inflammatory sICAM-1 in patients who are ventilated with high tidal volume indicate improved anti-inflammatory immune regulation in the low tidal volume group.
(3) Anaesthetic drugs modulated the alveolar immune response induced by one-lung ventilation in thoracic surgical patients. Desflurane decreased the expression of pro- and anti-inflammatory mediators. Propofol anaesthesia resulted in higher numbers of alveolar cells and a relative increase of the alveolar granulocyte fraction accompanied by a reduction of alveolar lymphocytes. The cytokine pattern followed the different recruitment of granulocytes into the alveoli during propofol and desflurane administration.

(4) The animal studies establish the one-lung ventilation-induced and surgery-related immune responses in comparison to those induced by conventional two-lung ventilation or spontaneous breathing in a single cohort. In contrast to the clinical studies and recently published data, which did not manage to separate the immune responses by ventilation from that after surgical manipulation, the data obtained from the porcine model of thoracic surgery demonstrate that one-lung ventilation and lung collapse/surgical intervention induce immune responses to nearly the same extent in the lungs of pigs. The lung injury is characterised by leukocyte recruitment and expression of pro-inflammatory mediators in the alveoli and in the systemic circulation.

(5) The cytokine release into the alveoli and the systemic circulation is reduced by the volatile anaesthetic desflurane by modulation of neutrophil activation in the lung and possibly in the peripheral blood.

(6) OLV and thoracic surgery increased the expression of pro-inflammatory IL8-mRNA in alveolar cells but not in lung tissue samples. The mRNA-expression was affected by propofol and desflurane anaesthesia to the same extent.
ACKNOWLEDGEMENTS

The clinical studies were performed at the operation theatre and intensive care units of the Department of Cardiovascular and Thoracic Surgery and the Department of Cardiology, Angiology and Pneumology of the Otto-von-Guericke-University Magdeburg, Germany. The experimental studies were done at the Department of Medical Sciences, Clinical Physiology, of the Uppsala University, Sweden. The samples obtained from patients and animals were analysed at the Institutes of Immunology and of Anatomy of the Otto-von-Guericke University Magdeburg, Germany.

I would like to express my gratitude to all who have contributed to the success of this work.

In particular, I wish to thank:

Göran Hedenstierna, for his mind-blowing enthusiasm, great generosity and kindness, and his profound knowledge not only in the field of lung physiology but also in general science. His open mind makes him the perfect mentor and the laboratory of Clinical Physiology in Uppsala a fantastic place to do scientific research.

Thomas Hachenberg, my teacher and mentor for continuously developing new ideas and sharing his vast knowledge and friendship with me. Without his inspiration, a lot of work would not have been done and many papers not been completed.

My fellow researcher Alf Kozian, who not only shared pigs and papers with me but also long evenings in the student hostel in Döbelnsgatan. The work could have never been completed without his tremendous support.

Moritz Kretzschmar, my co-worker in the laboratory for his valuable help and for improving English grammar and style in our manuscripts.
I wish to express my special thanks to the staff members of the Clinical Physiology Laboratory at Uppsala University, especially to:

_Eva-Maria Hedin_, for organisation, valuable help and professional assistance in handling bureaucracy. She brought some colour into the boring rooms of Döbelnsgatan, too.

_Agnete Ronéus, Karin Fagerbrink_ and _Anne Abrahamson_ for teaching, preparing and assisting in animal experiments. Above all, thank you for your vast kindness and for making the lab such a special place.

_Birgit Andersson_ and _Marianne Stoltenberg-Hansen_ for their important help in organisation and assistance in paper work.

_The staff members and co-workers of the Institutes of Anatomy and Immunology at the Otto-von-Guericke-University Magdeburg_ for their technical and organisational support in preparing and performing the sample measurements.

_My colleagues in the Cardiovascular and Thoracic Anaesthesia Department of the Otto-von-Guericke-University Magdeburg_ for their help and support, and for keeping the operation theatre running even though I was often absent and worked in different laboratories.

_My family_ for support, patience and understanding.

And above all, my beloved wife _Silvia_ and my sons, _Matthias and Paul_. You are the reason for everything in my life.

_Thomas Schilling, M.D., D.E.A.A._

_Uppsala, 2009, December_

***

The studies were supported by the Swedish Research Council (5315); the Swedish Heart and Lung Fund, and by institute sources of the Uppsala University and the Otto-von-Guericke-University Magdeburg.
REFERENCES


Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations
from the Faculty of Medicine 495

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)