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The Effects of One-Lung Ventilation on Cytokine Production from Lung Tissue of Rat Is One-Lung Ventilation Reasonable for Thoracic Surgery?

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Abstract We hypothesized that the collapsed lung during one-lung ventilation would be a main sourse of cytokine production. Rats were randomly assigned to 3 groups: control rats (C group) (n=10); one-lung ventilation rats (OLV group), ventilated by right lung (n=10); and two-lung ventilation rats (TLV group), ventilated after left thoracotomy (n=10). We measured interleukin (IL)-6, IL-10, and growth related oncogene/cytokine-induced neutrophil chemoattractant (GRO/CINC)-1 level in supernatants of lung tissue incubated for 6 hours. In the left lung (thoracotomy side), although IL-6 and IL-10 level from collapsed lung in the OLV group was higher than that in the C group, GRO/CINC-1 level was not. IL-6 and GRO/CINC-1 level from collapsed lung in the OLV group was lower than that in the TLV group, but level of IL-10 did not change. In the right lung (nonthoracotomy side), IL-6 and GRO/CINC-1 level in the OLV and TLV groups was higher than that in the C group, but IL-10 level did not change. With regard to balance of inflammatory and anti-inflammatory cytokine production, a completely collapsed lung on the thoracotomy side may be no more harmful than a ventilated lung in thoracotomy side. One-lung ventilation appears to be reasonable for thoracic surgery.

Introduction

Transthoracic esophagectomy with lymphadenectomy for esophageal cancer is well known to be one of the most stressful gastrointestinal operations to the patient, with the potential for a high operation-related rate of morbidity. Postoperative pulmonary complications such as respiratory failure are frequently observed[1].

Sakamoto et al.[2] reported that plasma levels of interleukin (IL)-6 after thoracic surgery, such as pneumonectomy and esophagectomy, were much higher than those after abdominal surgery, such as pancreaticoduodenectomy and colorectal resection. However, the mechanism of this increase in plasma IL-6 has not been fully clarified. Recent evidence revealed that IL-6 is produced by a variety of cell types in the lung, including alveolar macrophages, lung fibroblasts, endothelial cells, and airway epithelial cells under appropriate stimulation [3)-5)]. The transthoracic surgical approach is usually performed through one-lung ventilation (OLV) with the right lung collapsed. OLV involves the complete functional separation of the two lungs and is often the most important anesthetic consideration for patients undergoing thoracic surgery. The purpose of OLV in esophageal surgery is to achieve good surgical exposure, to allow aggressive lymphadenectomy around the upper mediastinum, and to allow thoracoscopic

mobilization of the esophagus[6]. A model of atelectasis in the rat showed that macrophages harvested from the lung 1 hour after atelectasis were upregulated with respect to IL-1 and tumor necrosis factor (TNF) production after lipopolysaccharide stimulation[7]. We reported that transthoracic esophagectomy causes an increase in IL-6 production from airway epithelial cells, secondary to increased expression of IL-6 mRNA, so that local response of lung tissue may be one source of increased serum IL-6 after esophagectomy in humans[8]. In that human study, we could neither evaluate the local cytokine production from the contralateral lung tissue in transthoracic esophagectomy, nor could we compare the collapsed lung with the ventilated lung on the thoracotomy side. We hypothesized that the collapsed lung during OLV would be a main source of cytokine production in rats. To clarify this hypothesis, we designed OLV and two-lung ventilation (TLV) animal models and compared production of cytokines such as TNFα, IL-1β, IL-6, growth-related oncogene/cytokine-induced neutrophil chemoattractant (GRO/CINC)-1, IL-10, and macrophage inflammatory protein (MIP)-2.

Materials and methods

Animals

Male Wistar rats (250-300 g) obtained from Japan SLC Inc. (Shizuoka, Japan) were used in this study. All animals were housed under controlled temperature (23±2℃) and humidity conditions with a 12-hour alternating light/dark cycle and free access to food and water. All experiments were conducted in accordance with the Committee on the Ethics of Animal Experiments in Yamaguchi University School of Medicine and carried out under the Guideline for Animal Experiments in Yamaguchi University School of Medicine and the Law (No. 105) and Notification (No. 6) of the Japanese Government.

Group allocation

The rats were divided into three groups: control rats without mechanical ventilation for baseline evaluation (C group) (n=10), OLV rats ventilated for 60 minutes via the right lung (OLV group) (n=10), and TLV rats ventilated for 60 minutes via bilateral lungs (TLV group) (n=10). Figure 1 illustrates the experimental protocol. Five rats in each group were used for cytokine assays and another five rats were used for histological study.

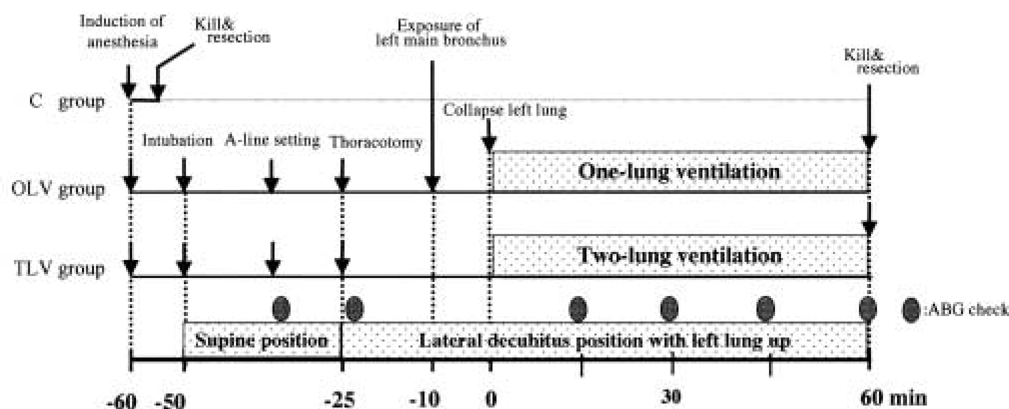

Figure 1. Study design and experimental protocol. ABG=Sampling of arterial blood, A line=arterial line, C=Control, OLV=one-lung ventilation, TLV=two-lung ventilation

Anesthesia and operation

After induction of anesthesia by 3.5% isoflurane and 60% N_2O in O_2, a polyethylene cannula (inner diameter, 1.5 mm) was inserted into the trachea. Anesthesia was mostly maintained with 1.5% isoflurane and 60% N_2O in O_2 throughout the experiment. The rats were ventilated with a small animal respirator (model No.141, NEMI Scientific Inc, Medway, MA) delivering a tidal volume of 8 ml/

kg body weight at 60/min. The femoral artery was cannulated with a polyethylene catheter (inner diameter, 0.5 mm) to continuously monitor systemic arterial pressure and to simultaneously withdraw samples for arterial blood gas analysis (ABL™ SYSTEM 625; Radiometer Medical A/S, Copenhagen, Denmark). The left femoral vein was cannulated with a polyethylene catheter for infusion of agents. The rats were continuously infused with lactated Ringer's solution at a rate of 2 ml/h. To eliminate artifacts caused by movement and to block spontaneous breathing movements, paralysis was induced with pancuronium bromide (0.2 mg bolus injection) and maintained with small doses. The respirator was set to deliver a fixed tidal volume of 8 ml/kg body weight at 60/min in the TLV group or a fixed tidal volume of 6 ml/kg body weight at 80/min in the OLVgroup, but the length of the dead space in the respiratory circuit in both groups was adjusted to keep the carbon dioxide tension at 35 to 40 mmHg. The airway pressure was continuously monitored. The expiratory tube was placed in the bottom of a water reservoir so that positive end expiratory pressure (PEEP) could be adjusted to 5 cmH_2O by changing the water level. Rectal temperature was monitored and maintained at 37°C with a small animal warmer with a thermometer (BWT-100: Bio Research Center Co., Ltd., Nagoya, Japan). All arterial pressure, airway pressure, and rectal temperature data were stored in a personal computer until analysis by Power Lab software (AD Instruments, Castle Hill, Australia).

The rat was placed in a right lateral position under the microscope (Leica, Wetzlow Germany) then the left lung was exposed by lateral thoracotomy in the 4th intercostal space. In the TLV group, the rat was ventilated for 60 min. In the OLV group, a good view of the hilar portion of the lung was established with the lung covered by wet gauze. For collapse of the lung, the left main bronchus was exposed and clamped by a small clip; however, the pulmonary artery and vein were preserved. To avoid overstretching the right lung during hilar exposure, ventilation was changed to a tidal volume of 6 ml/kg body weight at 80/min. In the OLV group, the rat was ventilated for 60 min with the left main bronchus clamped. Blood gas analysis was done at the following times: just after placement of the arterial line, after thoracotomy, and at 15 min, 30 min, 45 min, and 60 min after initiation of lung collapse. Initiation of a rate of 60/min in the TLV group was delayed 10 min according to mean time spent for hilar exposure. To help prevent small areas of atelectasis, a "sigh" consisting of twice the normal tidal volume was delivered by temporarily occluding the exhalation port of the ventilator[7]. To maintain mean arterial blood pressure above 90 mmHg, decreasing the concentration of isoflurane took priority over the infusion of lactated Ringer's solution. Animals were excluded from the study if their mean arterial pressure remained at less than 60 mmHg over 10 min after appropriate treatment. The rat was killed at the end of the each experiment by exsanguination from the descending aorta.

Specimens

In the C group, bilateral lungs were resected after intubation. In the OLV and TLV groups, bilateral lungs were obtained after completion of all procedures described above.

Lung tissue specimens were immediately cultured in 2 mL of Roswell Park Memorial Institute(RPMI)-1640 (GIBCO, Grand Island, NY) with 10% fetal calf serum (Whittaker Bioproducts Inc, Walkersville, MD) in an atmosphere of 95% air and 5% carbon dioxide at 37°C. After 6 hrs, supernatant was collected and stored at −80°C until assays. An incubation time of 6 hrs was decided by confirming in preliminary experiments that IL-6 levels in culture supernatants increased in a time dependent manner at 3, 6, and 12 hrs after incubation (data not shown).

Cytokine assays

We measured TNF-α, IL-1β, IL-6, GRO/CINC-1, IL-10, and MIP-2 concentrations in duplicate with commercially available enzyme-linked immunosorbent assay (ELISA) kit. (GRO/CINC-1; Immuno-Biological Laboratories, IBL Co., Ltd., Gunma, Japan; TNF-α, IL-1β, IL-6, IL-10 and MIP-2, BioSource International, Inc., Camarillo, CA) according to

the manufacturers' instructions. The lower detection limit for these kits is 4, 3, 2, 4.7, 5, and 1 pg/mL, respectively. Rat GRO/CINC is a peptide possessing biological activities analogous to those of the human IL-8 family[9]. Standard curves were prepared with recombinant cytokines run in conjunction with the samples. Optical density was determined at 450 nm with an automated plate reader (Multiskan MS, Thermo Labsystems Oy, Helsinki, Finland). Sample concentrations were determined by interpolation from the standard curve.

Histological study

The lung and heart were removed en bloc for histological examination; median sternotomy was accomplished, and the trachea was exposed after removal of the thymus. Animals were killed by bleeding from a cut aortic arch. The lungs were infused with 10% buffered paraformaldehyde via the trachea and maintained at a transpulmonary pressure of 20 cmH_2O for at least 3 hrs. Subsequently, the trachea was clamped, and the lungs were stored until histological examination. The lungs were sectioned along a vertical plane for tracheal axis (craniocaudal axis), and embedded in paraffin. Tissue sections of 5μm thickness were made, deparaffinized, and stained with hematoxylin and eosin. Much infiltration of inflammatory cells was seen externally under the visceral pleura rather than internally in the lung tissue, so we evaluated degree of inflammatory cell recruitment as follows. The number of infiltrating neutrophils in the alveolar region along the pleura (in a square field of 300×400 μm) of the three fields in a section were counted, and the average number of neutrophils per field was calculated, and examined in a blind manner. To avoid sampling bias, tissue samples from each rat were prepared at the same level below the carina for both lungs.

Statistical analysis

Values are expressed as mean±standard error (SE). Student t-test was used when differences between two groups were evaluated. Analysis of variance (ANOVA) was used with the Bonferroni-Dunn correction when differences between three groups were evaluated. A P value of < 0.05 was considered to be statistically significant.

Results

Blood gases and hemodynamics

Arterial pH and $PaCO_2$ did not differ significantly between the OLV and TLV groups throughout the experimental period (Table 1). All baseline values were similar for the two groups. After clamping left main bronchus, PaO_2 in the OLV group decreased sig-

Table I. Physiological variables at baseline and during 60 minutes ventilation

			Time after clamp			
	Baseline	Thoracotomy	15 min	30 min	45 min	60 min
Arterial pH						
OLV group	7.36±0.03	7.36±0.03	7.29±0.05	7.26±0.05	7.27±0.06	7.27±0.06
TLV group	7.35±0.01	7.36±0.02	7.34±0.04	7.31±0.03	7.31±0.02	7.30±0.03
PaO_2(torr)						
OLV group	176±9	164±10	79±4*†	83±4 *†	87±7 *†	86±5 *†
TLV group	171±11	160±13	185±11	162±16	170±19	170±15
$PaCO_2$(torr)						
OLV group	40±4	35±2	36±4	40±6	38±6	40±10
TLV group	42±3	38±4	39±5	42±7	43±6	42±8
Base Excess						
OLV group	-2.7±0.7	-5.1±1.9	-8.6±1.7*	-8.9±2.3*	-8.8±2.5	-9.0±3.1
TLV group	-3.4±1.0	-3.5±0.7	-5.0±0.8	-6.5±2.0	-5.2±1.8	-6.4±2.8

Baseline; blood sampled after arterial line setting. Thoracotomy; blood sampled after thoracotomy
OLV; one-lung ventilation (OLV), TLV; two-lung ventilation
*$p< 0.05$ vs. baseline value (within group);†$p < 0.05$ vs. TLV group, values are expressed as mean ± SEM.

nificantly. At 15 min and 30 min after clamping, lower base excess values were observed in OLV group than in the TLV group.

Maximal airway pressure did not differ significantly between OLV and TLV groups at any time during the course of the experiments (Figure 2). Mean arterial pressure decreased progressively in the OLV group from 25 min to 45 min after clamping, but it gradually returned to control level by the end of the experimental period.

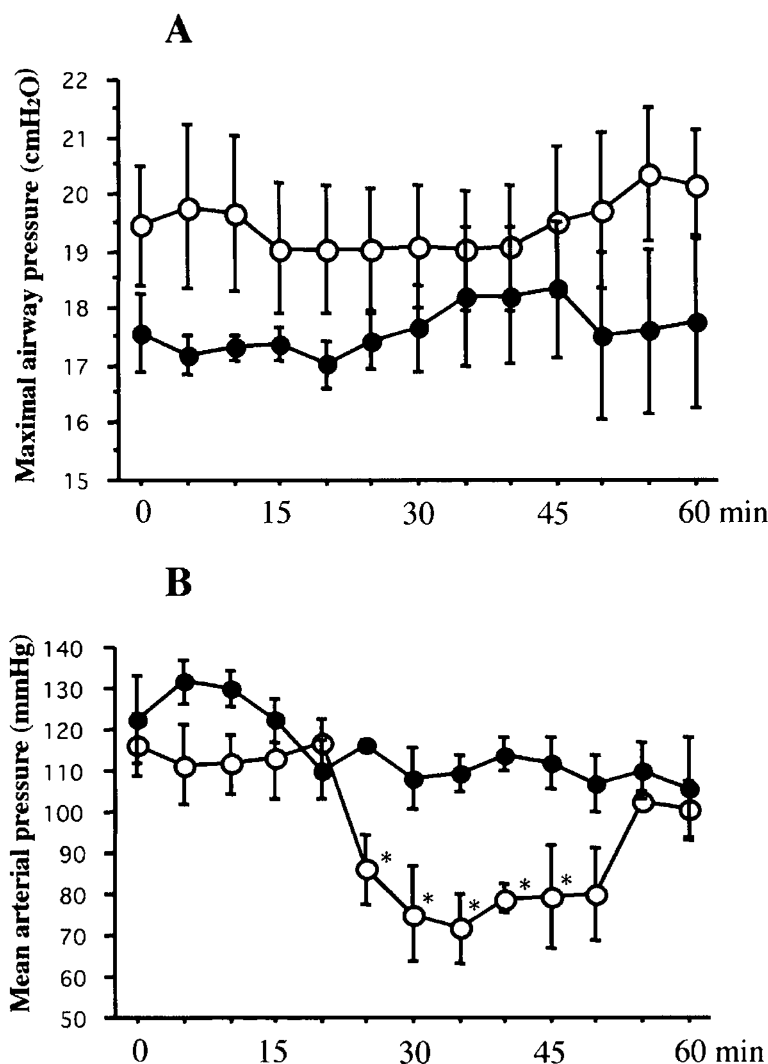

Figure 2. Maximal airway pressure (cmH_2O) (A) and mean arterial pressure (B) at baseline and after thoracotomy (mean±SEM). Open circles=OLV group; solid circles=TLV group. *$p < 0.05$; OLV group versus TLV group at the same time point.

Cytokine production in lung tissue

Levels of cytokine in the left lung are shown in Figure 3A, B, and C (IL-6, GRO/CINC-1, and IL-10, respectively), and those in the right lung are shown in Figure 3D, E, and F (IL-6, GRO/CINC-1, and IL-10, respectively). IL-6 levels of the left lung in the C group, OLV group, and TLV group were 1.5±0.3, 4.9±0.9, and 10.9±0.7 pg/ml/mg, respectively. GRO/CINC-1 levels of the left lung in the C, OLV, and TLV groups were 13.6±0.5, 12.8±2.7, and 25.5±3.1 pg/ml/mg, respectively. IL-10 levels of the left lung in the C, OLV, and TLV groups were 0.13±0.02, 0.27±0.04, and 0.20±0.01 pg/ml/mg, respectively. IL-6 levels of the right lung in the C, OLV, and TLV groups were 1.4±0.2, 4.1±0.7, and 4.6±0.7 pg/ml/mg, respec-

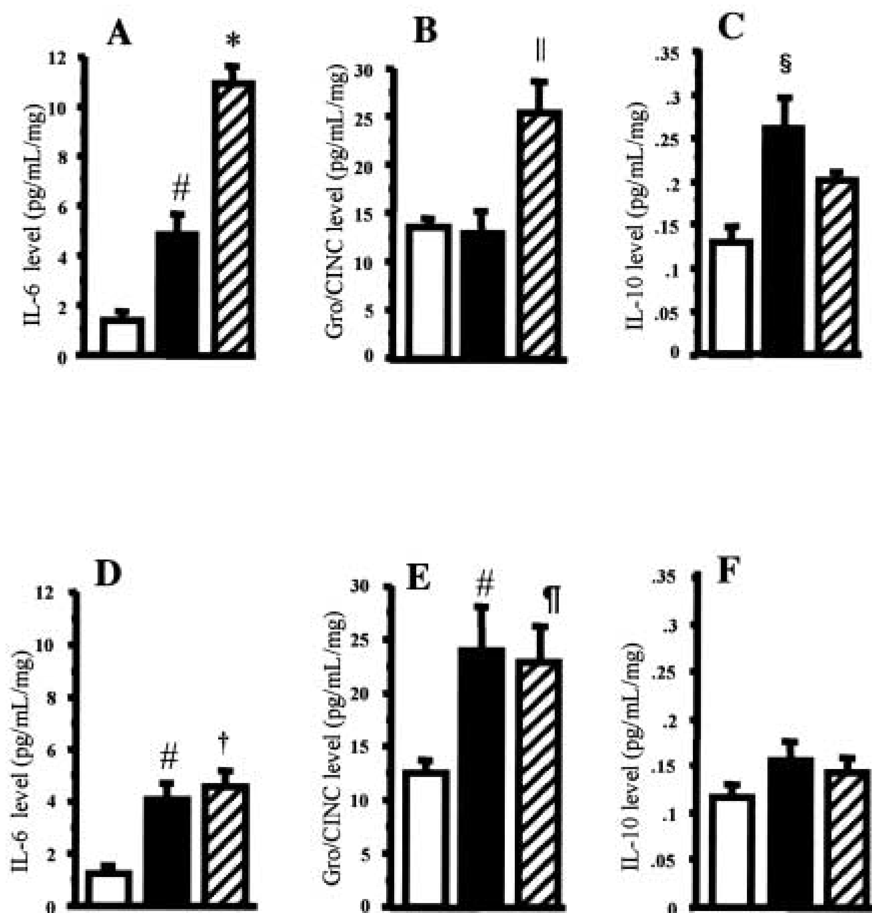

Figure 3. Cytokine levels produced from left lung tissue (thoracotomy side): (A) interleukin (IL)-6, (B) growth related oncogene/cytokine-induced neutrophil chemoattractant (GRO/CINC)-1, and (C) IL-10. Cytokine levels produced from right lung tissue (nonthoracotomy side): (D) IL-6, (E) GRO/CINC-1, and (F) IL-10. Open columns=C group; filled columns=OLV group; cross-hatched columns=TLV group. *p <0.0001; TLV group vs. C group and TLV group vs. OLV group. #p <0.05; OLV group vs. C group. ||p <0.01; TLV group vs. C group and TLV group vs. OLV group. §p <0.01; OLV group vs. C group. †p <0.01; TLV group vs. C group. ¶p <0.05; TLV group vs. C group.

tively. GRO/CINC-1 levels of the right lung in C, OLV, and TLV groups were 12.6±1.1, 23.9±4.3, and 23.0±3.4 pg/ml/mg, respectively. IL-10 levels of the right lung in the C, OLV, and TLV groups were 0.12±0.01, 0.16±0.02, and 0.15±0.01 pg/ml/mg, respectively.

Comparison between C group and OLV group: Production of IL-6 and IL-10 from the collapsed lung in the OLV group was significantly higher (p <0.05 and p <0.01, respectively) than that in the C group, but GRO/CINC-1 production was not significantly higher. Production of IL-6 and GRO-CINC-1 from the ventilated lung in the OLV group was significantly higher (p <0.05 and p <0.05, respectively) than that in the C group, but IL-10 production was not significantly higher.

Comparison of cytokine production between OLV and TLV in the ipsilateral lung: On the thoracotomy side, production of IL-6 and GRO/CINC-1 from the collapsed lung in the OLV group were significantly lower than those from the ventilated lung in the TLV group (p <0.0001 and p <0.01, respectively). IL-10 production from the collapsed lung in the OLV group was higher than that from

the ventilated lung in the TLV group, tending toward significance ($p < 0.07$). There was no difference between two ventilatory modes in production of IL-6, GRO/CINC-1, and IL-10 from lungs on the nonthoracotomy side.

Comparison of cytokine production between left and right lungs in the same ventilatory mode: In the OLV group, IL-10 production from the left (collapsed) lung was significantly higher than that from the right (ventilated) lung ($p < 0.05$), but IL-6 and GRO/CINC-1 production was not significantly higher. In the TLV group, Production of IL-6 and IL-10 from the left lung (thoracotomy side) was significantly higher than that from the right lung (nonthoracotomy side) ($p = 0.0002$ and $p = 0.009$, respectively), but GRO/CINC-1 production was not significantly higher.

Microscopic findings and neutrophil counts

Representative microscopic findings from the left lungs are shown in Figure 4. Structural alterations of the lung were not observed in either the OLV or TLV group in comparison with that of the C group. Examination of lung sections revealed the presence of cellular infiltration consisting mainly of neutrophils in the alveolar walls and spaces of subpleura in the left lung of the TLV group. Neutrophil count of infiltrate in the subpleural space in the left lung of the TLV group (29.2 ± 5.8 cells/field) was significantly increased over those of the C group (4.1 ± 1.7 cells/field, $p < 0.01$) and the OLV group (11.1 ± 4.2 cells/field, $p < 0.05$) (Figure 5A). No difference was observed among the groups in the right lung on the nonthoracotomy side: C group, 6.1 ± 3.0 cells/field; OLV group, 9.1 ± 2.2 cells/field; and TLV group, 9.8 ± 1.5 cells/field (Figure 5B).

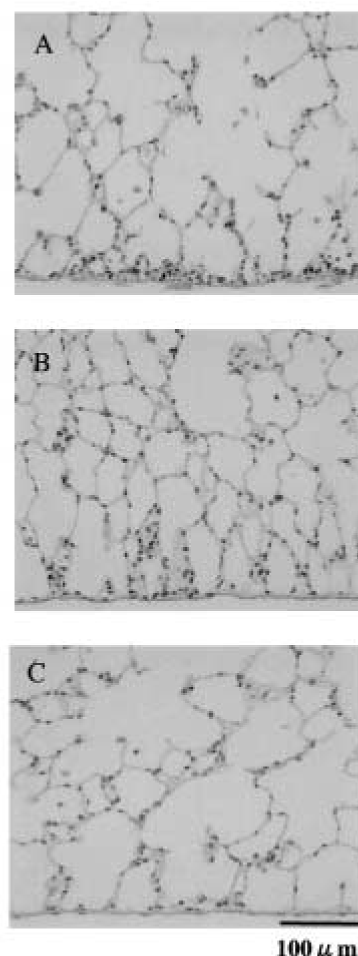

Figure 4. Representative light micrographs (hematoxylin and eosin, x 100) of subpleural tissue from each group. (A) Higher infiltration of neutrophils is seen in the left lung of the TLV group. (B) Moderate infiltration of neutrophils is seen in the left lung of the OLV group. (C) Less infiltration of neutrophils is seen in the left lung of the C group

Discussion

Our study is the first, to our knowledge, to indicate that production of IL-6 and GRO/CINC-1 from the collapsed lung in OLV after thoracotomy is suppressed more than that from the ventilated lung in TLV after thoracotomy and is similar to that from the contralateral lung in OLV. These results indicate that OLV in thoracic surgery may be a reasonable ventilatory strategy compared with TLV in open thoracotomy. The fact that less neutrophil infiltration was seen in collapsed lung during OLV than in ventilated lung during TLV support this conclusion.

In our small animal model, the ventilatory condition is a critical point. Thus, we performed a preliminary study to determine the optimum ventilatory condition. It is evident that a number of ventilatory strategies can produce or worsen lung injury[10]. The use of large tidal volumes[11], high peak airway pressures[12], high inspiratory flows[13], high

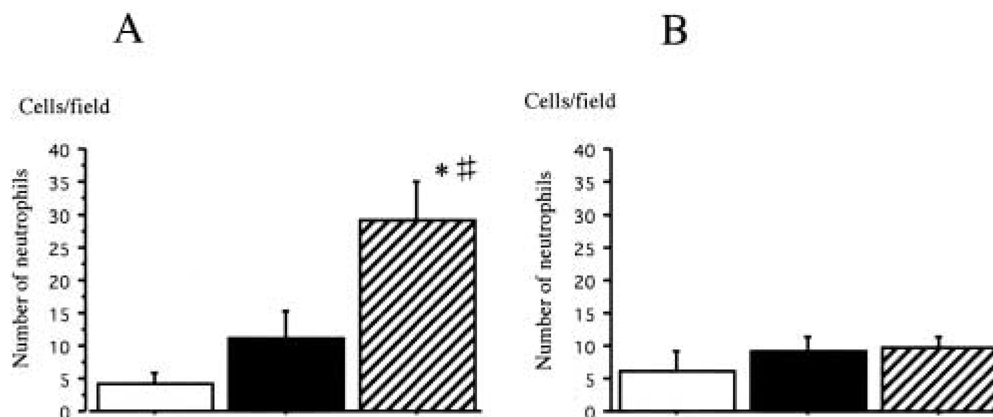

Figure 5. Comparison of neutrophil infiltration into the subpleural lung tissue. A=left lung; B=right lung. Open columns=C group; filled columns=OLV group; cross-hatched columns=TLV group. *p <0.01; TLV group vs. C group. #p <0.05; TLV group vs. OLV group

respiratory rates[14], and end-expiratory alveolar collapse with cyclic reopening[15] have all been proposed to play a role in the pathogenesis of ventilator-induced lung injury[16]. Ventilation with large tidal volumes causes the disruption of pulmonary epithelium and endothelium, lung inflammation, atelectasis, hypoxemia, and the release of inflammatory mediators[10),17)–21]. A tidal volume of 8 ml/kg body weight at a respiratory rate of 60/min in TLV or a tidal volume of 6 ml/kg body weight at 80/min in OLV were chosen as minimum tidal volumes on the basis of a series of preliminary experiments performed to observe blood gas analysis data (data not shown). Structural injury as a result of the shear forces generated by the repetitive opening and closing of distal small airways in the absence of PEEP may contribute to the production of inflammatory mediators[15]. It is therefore important to select an appropriate setting for ventilation with PEEP. Actually, many rats undergoing OLV in the absence of PEEP in the preliminary experiments were unable to stay alive until the end of the experiment due to severe acidosis and hypotension. Ventilation with about 5 cmH_2O PEEP appears to be essential for OLV in rat (data not shown).

Supplementation (or blocking) of various inflammatory cytokines has been found to induce (or abrogate) lung injury[18]. In light of the complexity and redundancy of the cytokine network, however, caution must be used in interpreting changes in levels of individual cytokines[18]. We therefore selected a variety of key inflammatory (TNFα, IL-1β, IL-6), chemotactic (GRO/CINC-1, MIP-2), and anti-inflammatory (IL-10) cytokines for examination. However, cytokine levels of TNFα, IL-1β, and MIP-2 were under detectable levels in the present model.

Local production of IL-6 may play a pivotal role in postoperative pulmonary function. We reported that transthoracic esophagectomy causes an increase in IL-6 production from airway epithelial cells, secondary to increased expression of IL-6 mRNA, so that local response of lung tissue may be one source of increased serum IL-6 after esophagectomy in humans[8]. The transthoracic approach is usually performed through OLV with the operative-side lung collapsed. Whether persistent collapse of the independent lung during thoracotomy influences production of cytokines such as IL-6, IL-8 and IL-10, or intensifies neutrophilic inflammation is unknown. We hypothesized that the inflammatory change occurring in collapsed lung in the OLV in the transthoracic approach must be taking place because of the high probability of postoperative pulmonary complication. In the present study, IL-6 production was intensified by OLV compared with that of the control, but no difference was seen between the left and right lungs. In opposition to our hypothesis, IL-6 production from the left lung in the TLV group was the most intensified of the three groups, showing a sig-

nificant difference from that in the right lung.

IL-8, a member of the CXC chemokine family, is produced by a wide variety of cells in the lung, including alveolar macrophages, bronchial epithelial cells, and pulmonary fibroblasts[22),24)]. IL-8 is one of the most potent chemoattractants for neutrophils[25)], and several groups of investigators have shown its augmented production in various systemic and lung diseases, such as acute respiratory distress syndrome (ARDS)[26)], inhalation injury[27)], expansion lung injury[28)], and acute exacerbation of idiopathic pulmonary fibrosis[24)]. In the present study, production of GRO/CINC, a murine homologue in the chemokine family related to humans, from left lung in the TLV group was found to be significantly higher than that in the C and OLV groups. Correspondingly, neutrophil counts in the left lungs in the TLV group were increased compared with those in the C and OLV groups. However, neutrophil counts in the right lungs (nonthoracotomy side) in the OLV and TLV groups were not increased in spite of high levels of GRO/CINC and low levels of IL-10. It appears that thoracotomy itself may also influence neutrophil infiltration.

IL-10 is an anti-inflammatory cytokine that is produced initially in sepsis and plays a role in abating IL-1α, IL-1β, TNF-α, IL-6, IL-8, nitric oxide (NO), and MIP-2 production[29)−32)]. IL-10 is produced by monocytes, macrophages, T cells, B cells, and epithelial cells[29)−31)]. Stimulating the counter-inflammatory response, such as by stimulating the production of IL-10, may decrease inflammatory response and attenuate organ injury[33)−35)]. In the present study, IL-10 production from the left lung in the OLV group (collapsed lung) was highest among the three groups, possibly reflecting the suppression of IL-6 and GRO/CINC-1 production from the left lung in the OLV group. The protective effect of IL-10 in animal models is controversial because of the negative results reported by two independent investigators[26),37)]. In our animal model, protective effect may be gained by collapse of the lung itself, but further investigation of survival results after OLV will be needed to clarify clinical meanings. Decreased neutrophil infiltration into the collapsed lung in the OLV group may support protective aspects of lung collapse.

With regard to balance of inflammatory and anti-inflammatory cytokine production, lung tissue may be one potential source of cytokines after thoracic surgery, but a completely collapsed lung on the thoracotomy side may be no more harmful than a ventilated lung in thoracotomy side. OLV appears to be a reasonable method of ventilatory management during thoracic surgery.

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