Anesthesiology
1999; 91:187-97

Volatile Anesthetics Augment Expression of Proinflammatory Cytokines in Rat Alveolar Macrophages during Mechanical Ventilation

Naoki Kotani, M.D., Satoshi Takahashi, M.D.,* Daniel I. Sessler, M.D.,† Eiji Hashiba, M.D.,‡ Takeshi Kubota, M.D.,* Hiroshi Hashimoto, M.D.,* Akitomo Matsuki, M.D.§*

Background: **Previous studies indicate that anesthesia and surgery induce an inflammatory reaction in alveolar macrophages. However, they failed to independently evaluate the relative contributions of factors including mechanical ventilation, general anesthesia, and surgical stress. Therefore, the authors tested the hypothesis that inflammatory reactions at the cellular level in alveolar macrophages are induced within 2 h of inhalation of volatile anesthetics under mechanical ventilation.**

Methods: **After administration of pentobarbital, rats were allocated to the nonventilated control or spontaneous or mechanical ventilation (n = 15/group) for 2 h at a fraction of inspired oxygen ($F_{I_{O_2}}$) of 0.21. In a separate series of experiments, rats were mechanically ventilated without volatile anesthesia, or during exposure to halothane, enflurane, isoflurane, or sevoflurane (n = 15/group). Pulmonary lavage was performed, and RNA was extracted from harvested cells. The mRNA for the proinflammatory cytokines interleukin (IL)-1α, IL-1β, IL-6, macrophage inflammatory protein-2 (MIP-2), interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) were measured by semiquantitative reverse transcription-polymerase chain reaction using β-actin as an internal standard. Pulmonary lavage concentrations of these cytokines were measured by enzyme-linked immunoassay.**

Results: **The lavage cell count and cytology were similar in each series of the experiment. Gene expression of MIP-2 and TNF-α was greater during mechanical than spontaneous ventilation and nonventilation control. However, the concentrations of cytokines except MIP-2 and TNF-α were less than detection levels. During exposure to volatile anesthetics, gene expression for IL-1β, MIP-2, IFN-γ, and TNF-α all increased significantly compared with mechanical ventilation alone. Significant increases in lavage concentrations of MIP-2 and TNF-α were also observed.**

Conclusions: **Gene expression of proinflammatory cytokines increase after inhalation of volatile anesthetics under mechanical ventilation. These data indicate that inhalation of volatile anesthetics under mechanical ventilation induces an inflammatory response at the transcriptional level within 2 h. (Key words: Inflammation; interferon; interleukin; macrophage inflammatory protein; tumor necrosis factor.)**

ALVEOLAR macrophages are the first line of pulmonary defense. In previous studies, we reported that an influx of neutrophils and alveolar macrophage aggregation increased as the duration of anesthesia and surgery increased.[1,2] Macrophage aggregation and influx of neutrophils are induced by various immunomodulators and characterize inflammatory reactions in the lungs.[3-5] Interestingly, these responses were minimal within 2 h of anesthesia and surgery. Although neutrophil influx and alveolar macrophage aggregation were minimal within 2 h of anesthesia and surgery, it is likely that an inflammatory reaction is induced at cellular levels in alveolar macrophages, such as gene expression and production of proinflammatory cytokines.

* Assistant Professor, Department of Anesthesiology, University of Hirosaki School of Medicine.

† Professor, Department of Anesthesia and Perioperative Care, University of California, San Francisco; Professor, Ludwig Boltzmann Institute for Clinical Anesthesia and Intensive Care; Director of Outcomes Research, Professor, and Vice-Chair, Department of Anesthesia and General Intensive Care, University of Vienna.

‡ Resident, Department of Anesthesiology, University of Hirosaki School of Medicine.

§ Professor and Chair, Department of Anesthesiology, University of Hirosaki School of Medicine.

Received from the Outcomes Research Group™; the Department of Anesthesiology, University of Hirosaki, Hirosaki, Japan; the Department of Anesthesia and Perioperative Care, University of California, San Francisco, California; the Ludwig Boltzmann Institute for Clinical Anesthesia and Intensive Care, Vienna, Austria; and the Department of Anesthesia and General Intensive Care, University of Vienna, Vienna, Austria. Submitted for publication August 21, 1998. Accepted for publication February 15, 1999. Supported by a grant-in-aid for Scientific Research, 08457399, Department of Education, Tokyo, Japan; National Institutes of Health grant GM49670, Bethesda, Maryland; the Joseph Drown Foundation, Los Angeles, California; and the Fonds zur Förderung der Wissenschaftlichen Forschung, Vienna, Austria. Major corporate funding for the Outcomes Research Group is provided by Augustine Medical, Inc. The authors do not consult for, accept honoraria from, or own stock or stock options in any anesthesia-related company.

Address reprint requests to Dr. Kotani: Department of Anesthesiology, University of Hirosaki School of Medicine, Hirosaki 036, Japan. Address electronic mail to: nao@cc.hirosaki-u.ac.jp. On the worldwide web: outcomes-research.org

Our previous human studies did not independently evaluate the relative contributions of factors including mechanical ventilation, general anesthesia, and surgical stress. We believe that each of these factors may contribute significantly to observed intraoperative pulmonary inflammatory responses. Surgical stress obviously induces a generalized inflammatory response.[6] The effects of volatile anesthetic inhalation during mechanical ventilation is also of interest, because our previous study showed that macrophage aggregation is more pronounced during isoflurane than propofol anesthesia.[2] Finally, it is well established that mechanical ventilation can augment inflammatory reactions in the injured lungs.[7]

To evaluate the relative contribution of anesthesia to the inflammatory response of alveolar macrophages, we tested the hypothesis that inflammatory reactions at cellular levels of alveolar macrophages are induced within 2 h of inhalation of volatile anesthetic under mechanical ventilation. In addition to measuring concentrations of proinflammatory cytokines in pulmonary lavage fluid, we used the reverse transcription polymerize chain reaction (PCR) to amplify mRNA of proinflammatory cytokines and detect changes in alveolar macrophages at the transcriptional level. Given the complexity and redundancy of the cytokine network,[8,9] we evaluated the key proinflammatory cytokines, including interleukin (IL)-1α, IL-1β, IL-6, macrophage inflammatory protein-2 (MIP-2), interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α).

Methods

The study protocol was approved by the Institutional Animal Care Committee at the University of Hirosaki. One hundred twenty Wistar male rats weighing approximately 250 g were studied. All rats were maintained under standard conditions with free access to water and rodent laboratory food. Artificial light was present from 7 A.M. to 7 P.M. each day, and room temperature was maintained between 22°C and 24°C. The experiments in each group for each study were performed at randomly selected times ranging from 9 A.M. to 5 P.M.

Animal Preparation

After the animals were anesthetized with 50 mg/kg intraperitoneal pentobarbital, the trachea was excised and a catheter (Angiocath, 16 gauge; Deseret Medical, Sandy, UT) was inserted into the trachea. Catheters (Angiocath, 24 gauge) were also inserted into the femoral artery and vein. Lactated Ringer's solution was infused at a rate of 10 ml · kg^{-1} · h^{-1} through the venous catheter. We continuously monitored mean arterial pressure and heart rate from the femoral arterial catheter. The rats were placed on a heating pad (Small Animal Warmer BWT-100; BRC Co., Nagoya, Japan) and rectal temperature was maintained between 37°C and 37.5°C.

Experimental Protocol: Ventilation Modes

The purpose of this experiment was to evaluate gene expression and production of proinflammatory cytokines after spontaneous and mechanical ventilation. Forty-five rats were equally assigned to one of three groups: The control group underwent neither spontaneous nor mechanical ventilation. That is, they were killed for pulmonary lavage (n = 10) and to determine wet-to-dry ratios (n = 5) immediately after induction of pentobarbital anesthesia and tracheostomy. A second group of animals (n = 15) was mechanically ventilated for 2 h, whereas rats in the third group (n = 15) breathed spontaneously during this period.

The mechanically ventilated rats were paralyzed by a continuous infusion of 0.1 ml · kg^{-1} · h^{-1} vecuronium. A rodent ventilator (model 683; Harvard Apparatus, South Natick, MA) set to 10 ml/kg was used in the mechanically ventilated animals. Arterial blood was sampled for analysis of pH, oxygen tension, and carbon dioxide tension at the beginning of each experiment (initial values) and again 2 h later (final values) except in the control group. The respiratory rate was controlled to produce an initial arterial carbon dioxide tension between 35 and 45 mmHg. Two hours after the start of the experiments, 10 rats in the ventilated groups were killed for whole pulmonary lavage, and the remaining five rats were used to evaluate the wet-to-dry weight ratios and histopathologic changes that occurred in the lungs.

Experimental Protocol: Volatile Anesthetic Exposure

The purpose of the experiment was to evaluate the effect of volatile anesthetic on alveolar macrophages in mechanically ventilated rats. We assigned 75 rats to one of five groups of 15 animals: One group was mechanically ventilated with continuous infusion of 0.1 mg · kg^{-1} · h^{-1} vecuronium. The remaining animals were mechanically ventilated with 1.5 minimum alveolar concentration halothane (1.6%), enflurane (3.4%), isoflurane (2.1%), or sevoflurane (3.7%) supplemented with continuous infusion of 0.1 mg · kg^{-1} · h^{-1} vecuronium. The rodent ventilator was set as described before in the

ventilation modes protocol. Arterial blood was sampled for analysis of pH, oxygen tension, and carbon dioxide tension immediately after the start of the experiment in the control group or immediately after the start of inhalation of volatile anesthetics in the remaining four groups (initial values) and again 2 h later (final values). Ten and five rats in each group were used for whole pulmonary lavage and histopathologic examination of the lungs, respectively.

Histopathologic Examination and the Wet-to-dry Ratios

Immediately after the rats were killed, we measured pulmonary wet:dry ratios to evaluate pulmonary congestion. The right upper lobe was weighed and then dried to a constant weight at 60°C for 24 h.

The lower lobe of the right lung was fixed by the instillation of 10% formaldehyde solution through the right lower bronchus at a pressure of 20 cm water. The fixed lung was embedded and hematoxylin and eosin and Mallory-Azan stains were applied. We graded the degree of the lung injury using a five-point scale according to a combined assessment of alveolar and interstitial edema, hemorrhage, and accumulation and aggregation of immune cells based on a previously reported system: 0 = minimum damage, 1 = mild damage, 2 = moderate damage, 3 = severe damage, and 4 = maximum damage.[10]

Whole Pulmonary Lavage and Cell Isolation

Whole pulmonary lavage was performed in 10 rats from each group. Five milliliters saline solution, 0.9%, containing 16 mM lidocaine hydrochloride titrated with NaOH to a pH of 7.4 was instilled gently *via* a syringe and then withdrawn. This procedure was repeated 10 times, so that 50 ml of the saline solution was instilled. Three-milliliter aliquots of lavage fluid were reserved for total cell count, viability, and cell differentiation and aggregation. The rest was centrifuged immediately at 200*g* for 10 min. After the supernatant was decanted to measure cytokine concentrations, the cell pellets were dissolved in 0.5 ml of guanidinium buffer solution (4 M guanidinium isothiocyanate, 50 mM Tris HCl, 10 mM EDTA, 2% sarcoryl, and 100 mM mercaptoethanol).

The total cell number was determined using a hemocytometer. The viability of alveolar cells was evaluated by an ability to exclude the 0.2% trypan blue, as described in our previous studies.[1,2] Cell differentiation and aggregation were examined by counting 500 cells on a Wright-Giemsa-stained slide.

RNA Isolation and cDNA Synthesis

RNA was isolated from the guanidinium buffer by the well-established acid guanidinium-phenol-chloroform method.[11] The amount of isolated RNA was measured using a spectrophotometer (model DU-65; Beckman, Tokyo, Japan). We obtained 2.4–3.8 μg RNA from each sample. By incubation at 40°C for 60 min, cDNA was synthesized from 2 μg RNA with 20 μl total reaction mixture, including Tris-HCl buffer (pH 8.3), 1 mM dNTPs, and 0.125 μM oligo dT primers, and 20 U RNase inhibitor and 0.25 U AMV reverse transcriptase. After 60 min of incubation, the reverse transcriptase was inactivated at 95°C for 5 min.

The Semiquantitative Reverse Transcription Polymerase Chain Reaction

The reverse transcription polymerase chain reaction (PCR) mixture (50 μl) contained cDNA synthesized from 0.2 μg RNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl2, 0.2 mM dNTP, 0.2 μM 5' and 3'oligonucleotide primers, and 2.5 U Taq polymerase (Takara, Co., Tokyo, Japan).

The reaction mixture was amplified in a DNA thermocycler (Perkin-Elmer Co., Irvine, CA). Each cycle consisted of denaturation at 94°C for 1 min, annealing at 56°C (for IL-6 and IFN-γ) or 59°C (for other cytokines) for 1 min, and extension at 72°C for 1 min. The optimal number of PCR cycles for each primer set was determined in preliminary experiments so that the amplification process was performed during the exponential phase of amplification.[12] The number of PCR cycles are as follows; 27 for β-actin, 32 for IL-1α, 31 for IL-1β, 35 for IL-6, 28 for IFN-γ, 33 for MIP-2, and 28 for TNF-α. The sequence of cytokine-specific primer pairs, 5' and 3', is shown in table 1. Coamplification of the cDNA for each cytokine and β-actin was performed in single tubes. The β-actin primers were added after several cycles with only cytokine primer so that the final number of PCR cycles was optimal for both the cytokine and β-actin.

The PCR products were quantified by densitometry measurements. The PCR products were separated by electrophoresis on a 1.8% agarose gel containing 0.5 μg/ml ethidium bromide. The PCR products were visualized on a transilluminator (model FBTIV-816; Fisher Scientific, Pittsburgh, PA) at 312 nm wavelength and photographed with Polaroid 667 film (Japan Polaroid, Tokyo, Japan). The band images were obtained by scanning the image with a ScanJet 3P (Hewlett-Packard, Andover, MA). The total intensity (average intensity × total pixels) of each band was measured using Mocha soft-

Table 1. Sequence of Cytokine-specific Primer Pairs

β-actin (764 bp)	Sense	TTGTAACCAACTGGGACGATATGG
	Antisense	GATCTTGATCTTCATGGTGCTAGG
IL-1α (623 bp)	Sense	CTAAGAACTACTTCACATCCGCAGC
	Antisense	CTGGAATAAAACCCACTGAGGTAGG
IL-1 (752 bp)	Sense	TCCTTGTGCAAGTGTCTGAA
	Antisense	GAGAGGTGCTGATGTACCAG
IL-6 (614 bp)	Sense	CAAGAGACTTCCAGCCAGTTGC
	Antisense	TTGCCGAGTAGACCTCATAGTGACC
MIP-2 (287 bp)	Sense	GGCACATCAGGTACGATCCAG
	Antisense	CTTCAGTTGGGAACCGTCCCA
IFNα (288 bp)	Sense	ATCTGGAGGAACTGGCAAAAGGACG
	Antisense	CCTTAGGCTAGATTCTGGTGACAGC
TNFα (295 bp)	Sense	TACTGAACTTCGGGGTGATTGGTCC
	Antisense	CAGCCTTGTCCCTTGAAGAGAACC

ware (Jandel Scientific Software, San Rafael, CA). To evaluate the relative amount of cytokine mRNA in each rat, the cytokine-to-β-actin ratio of the intensity of ethidium bromide luminescence for each PCR product was calculated.[13]

The Cytokine Enzyme-linked Immunoassay in Whole Pulmonary Lavage Fluid

Cytokine analysis on the whole pulmonary lavage fluid (IL-1α, IL-1β, IL-6, MIP-2, IFN-γ, TNF-α) was performed in duplicate in a blinded manner using commercially available enzyme-linked immunoassay kits (TFB Co., Tokyo, Japan). The minimum detection levels were 5, 5, 31, 1, 7, and 0.7 pg/ml, respectively. In our laboratory, the intra- and interassay coefficients of variation of each cytokine measurement are less than 5% and 8%, respectively. All kits except IL-1α were specific for the rat. The kit for IL-1α was a murine preparation having suitable cross-reactivity with rat IL-1α. The absorbance of each well was read at 450 nm using a microplate reader. Background absorbency of blank wells was subtracted from the standard and unknowns before the sample concentration was determined.

Statistical Analyses

We used nonparametric methods for statistical analyses. Wilcoxon signed-rank, Mann-Whitney U, and Kruskal-Wallis tests were used when appropriate. Data are expressed as mean ± SD; $P < 0.05$ was considered significant.

Results

Ventilation Modes

Table 2 shows changes in cardiorespiratory parameters in the ventilation modes protocol. The mean arterial pressure and heart rate did not change over time in the spontaneous and mechanical ventilation groups. Although the inhaled oxygen fraction was only 0.21, the arterial oxygen pressure was more than 80 mmHg in all animals; arterial oxygen pressure and pH also did not change over time or among groups. Although the arterial carbon dioxide tension decreased slightly at the final time point in animals in the spontaneous ventilation group, differences between spontaneous and mechanical ventilation were not observed. There were no differences in recovery rate, total cell number, cell distribution, aggregation, viability or wet-to-dry ratios among the three groups. Histologic changes in the lungs were minimal in all the rats studied (table 3).

The relative concentrations of mRNA for MIP-2 and TNF-α in the pulmonary lavage fluid were significantly greater in the animals undergoing mechanical ventilation than in those without ventilation ($P < 0.05$), whereas there was no significant difference between the nonventilated controls and the spontaneous ventilation group (fig. 1). The concentration of IL-1α, IL-1β, IL-6, and IFN-γ were less than the detection level in pulmonary lavage fluid. None of the other pulmonary lavage cytokine concentrations differed among the three groups (fig. 2).

Volatile Anesthetic Exposure

Table 4 shows the changes in cardiorespiratory parameters in each group. Both in the initial and final time points, mean arterial pressure and heart rate were significantly less in rats exposed to volatile anesthetics than in the control rats that were mechanically ventilated without volatile anesthesia. Core temperature, pH, respiratory rate, and the partial pressures of oxygen and carbon dioxide in arterial blood did not differ significantly with time or among groups. There were no differences in cell recovery rate, total cell number, cell distribution, aggre-

Table 2. Cardiorespiratory Responses and Body Temperature during Spontaneous and Mechanical Ventilation

	Time	Control	Spontaneous Respiration	Mechanical Ventilation
MAP (mmHg)	Initial	100 ± 4	102 ± 6	101 ± 7
	Final		100 ± 6	101 ± 5
HR (bpm)	Initial	411 ± 15	423 ± 22	423 ± 21
	Final		424 ± 22	426 ± 23
T_{core} (°C)	Initial	37.0 ± 0.2	37.1 ± 0.2	37.1 ± 0.2
	Final		37.0 ± 0.2	37.1 ± 0.3
Respiratory rate (rate/min)	Initial	29 ± 2	29 ± 2	30 ± 3
	Final		30 ± 3	32 ± 3*
*p*H	Initial	7.41 ± 0.02	7.41 ± 0.02	7.43 ± 0.02
	Final		7.41 ± 0.02	7.42 ± 0.03
Pa_{CO_2} (mmHg)	Initial	40 ± 2	39 ± 1	39 ± 2
	Final		37 ± 1*	38 ± 1
Pa_{O_2} (mmHg)	Initial	97 ± 9	98 ± 9	98 ± 8
	Final		98 ± 10	100 ± 6

T_{core} = rectal temperature; RR = respiratory rate.

Values are mean ± SD. Initial values identify the beginning of the study (immediately after induction of pentobarbital anesthesia and tracheostomy); final values are those obtained 2 h later. There are no final values in the control animals because they were killed at the beginning of the study. There were no statistically significant differences among groups.

* Statistically significant difference from initial values.

gation, or viability among the groups. In all animals, histologic changes were minimal and did not include edema, hemorrhage, or infiltration of immune cells into alveolar spaces. We also failed to observe differences in the wet-to-dry ratios among the groups (table 5).

The relative amount of mRNA for IL-1β and MIP-2 increased significantly in all groups with volatile anesthetics compared with the control group that was not given volatile anesthesia. The relative amount of mRNA for TNF-α was significantly greater than control in the rats given halothane, enflurane, or isoflurane. Furthermore, IFN-γ mRNA increased significantly in the groups given halothane or enflurane (fig. 3).

Table 3. Alveolar Cells and Pulmonary Histopathologic Changes during Spontaneous and Mechanical Ventilation

	Control	Spontaneous Respiration	Mechanical Ventilation
Recovery rate (%)	84 ± 3	84 ± 4	86 ± 3
Cell concentration ($\times 10^5$/cm^3)	1.5 ± 0.3	1.6 ± 0.3	1.5 ± 0.3
Total cell ($\times 10^6$)	6.4 ± 1.2	6.6 ± 1.4	6.5 ± 1.3
Macrophage (%)	96.4 ± 2.0	97.3 ± 1.8	96.7 ± 1.3
Lymphocyte (%)	2 ± 1.3	1.2 ± 1.2	1.7 ± 0.9
Neutrophil (%)	1.6 ± 1.0	1.5 ± 1.1	1.6 ± 0.5
Aggregation (%)	2.3 ± 2.6	2.4 ± 2.1	2.8 ± 1.7
Viability (%)	98 ± 2	98 ± 2	98 ± 1
Histology (0–4)	0 (0–0)	0 (0–1)	0 (0–0)
Wet/dry ratio	4.8 ± 0.1	4.9 ± 0.2	4.8 ± 0.2

Data are mean ± SD, except for the histologic score, which is expressed in terms of median (range). There were no significant differences among the groups.

Figure 4 shows the cytokine concentrations in the pulmonary lavage fluid. As in the ventilation modes protocol, IL-1α and IL-6 were undetectable. The level of MIP-2 in pulmonary lavage fluid was significantly greater in the groups given halothane, enflurane, and isoflurane than in those without volatile anesthesia. The concentrations of TNF-α in the lavage fluid were also significantly greater in the groups given halothane or enflurane than in those without volatile anesthetics. The concentrations of IL-1β and IFN-γ in the pulmonary lavage fluid did not differ significantly among the groups (fig. 4).

Discussion

Mechanical ventilation induced a slight increase in gene expression of the proinflammatory cytokines MIP-2 and TNF-α. In our study, carbon dioxide tension and respiratory rate did not differ between spontaneous and mechanical ventilation. The contributions of pentobarbital and tracheostomy to this response were minimal, because there were no differences in message pattern or cytokine production between spontaneous ventilation and the control groups. Our results thus suggest that positive airway pressure is the most

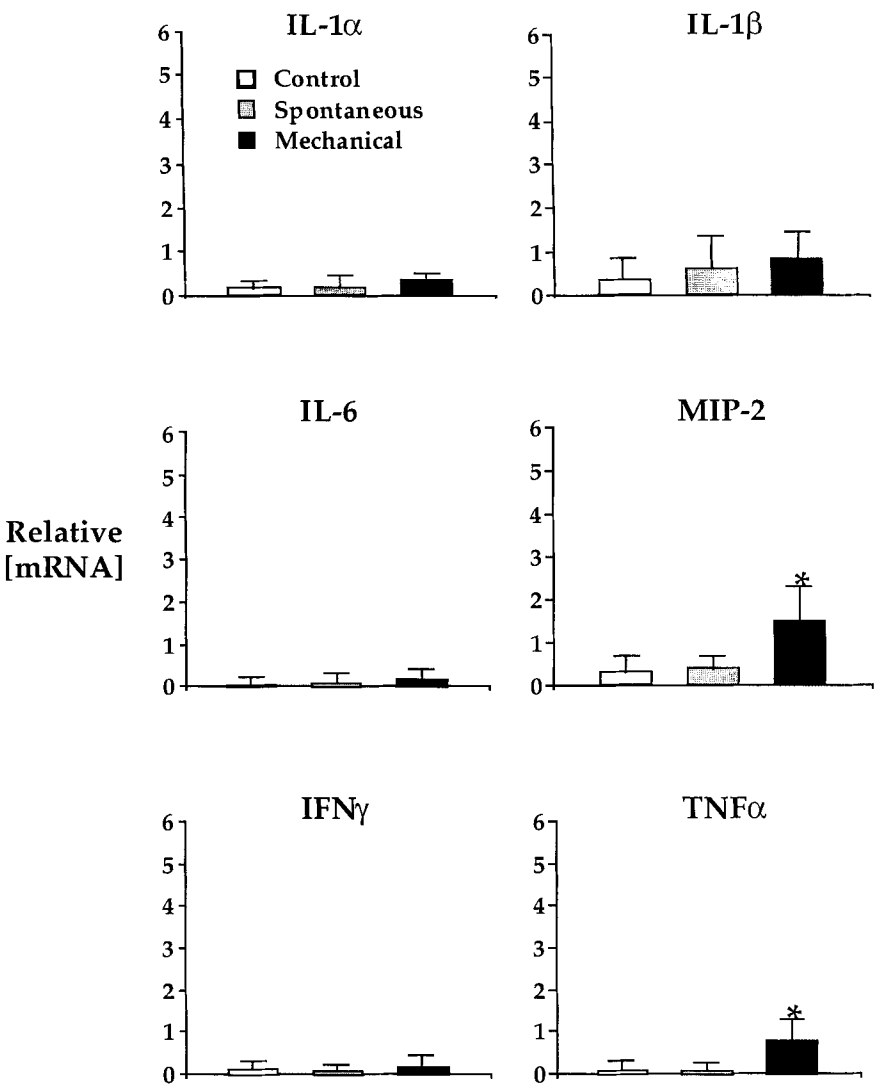

Fig. 1. Changes in expression of proinflammatory cytokines during spontaneous and mechanical ventilation. Values are expressed by the ratio of cytokine mRNA to β-actin mRNA and are presented as mean ± SD. Asterisks indicate statistically significant differences ($P < 0.05$) from spontaneous ventilation.

likely factor contributing to the observed increase in gene expression.

The marked increase in gene expression of TNF-α has been reported during mechanical and high-frequency ventilation in a surfactant-deficient rabbit model.[14] Our study showed that an increase in gene expression of proinflammatory cytokines also occurs in the normal uninjured lungs. Although the exact mechanism of the increase in MIP-2 gene expression cannot be determined from our data, one study showed that gene expression of MIP-2 increased faster than did gene expression for the other proinflammatory cytokines.[15]

The notable finding in this study is that expression of proinflammatory cytokines increased after 2 h of inhalation of volatile anesthetics during mechanical ventilation. It is well established that alveolar macrophages react mainly to inhaled substances. For example, gene expression for proinflammatory cytokines increases after inhalation of noxious substances such as aerosolized lipopolysaccharide or bacteria,[15–17] nitrogen dioxide,[18] ozone,[19] and even 100% oxygen.[20] Our results, together with previous studies, thus strongly suggest that an inflammatory reaction is induced at the transcriptional level within 2 h of exposure to volatile anesthetics under mechanical ventilation.

It is interesting that both the expression and concentration of MIP-2 increased simultaneously. MIP-2 is one of the most potent chemoattractants for neutrophils to the distal airway. For example, tracheal instillation of

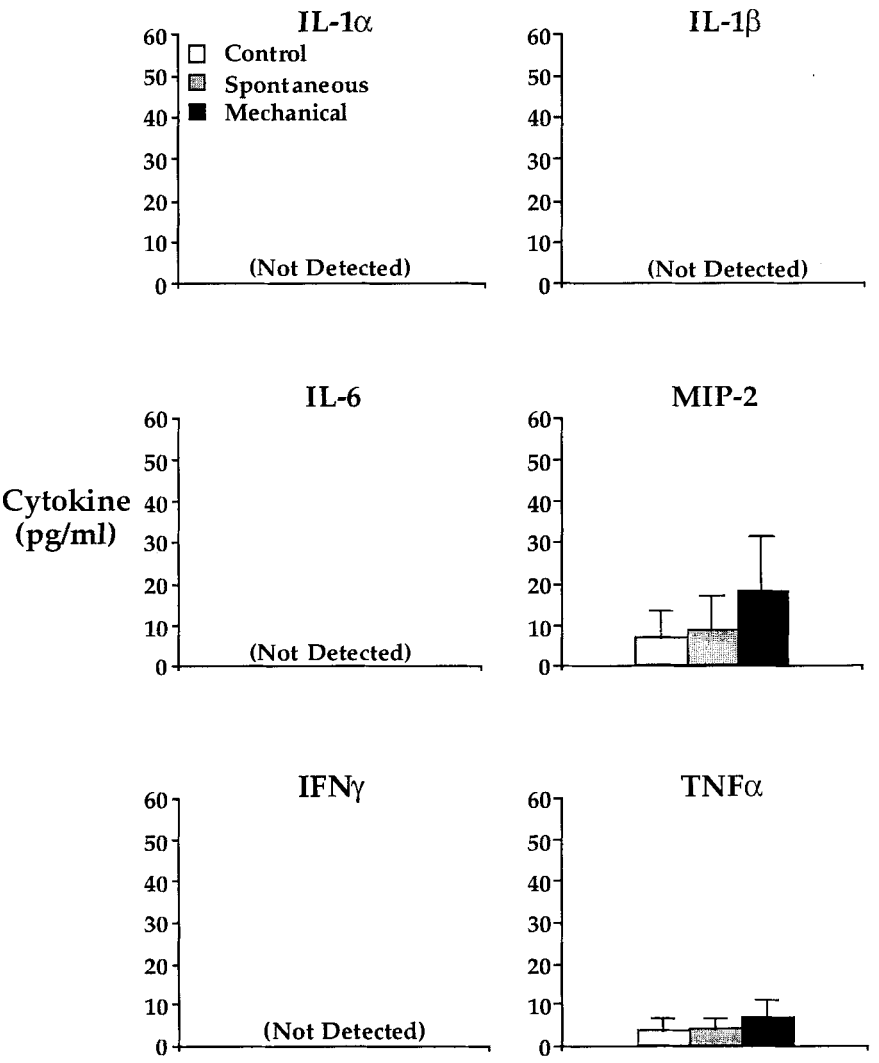

Fig. 2. Changes in the absolute pulmonary lavage concentration of proinflammatory cytokines during spontaneous and mechanical ventilation. Values are expressed as mean ± SD. There were no significant differences between the groups.

recombinant rat MIP-2 causes neutrophil accumulation 2 h after administration.[15] We did not observe an increased number of neutrophils in the pulmonary lavage fluid. This result is consistent with our previous study in which neutrophil influx was not observed until 4 h after anesthesia and surgery. Ulich *et al.*[21] observed neutrophil influx to the distal airway 4 - 6 h after administration of lipopolysaccharide.

We also observed increases in both the expression and concentration of TNF-α 2 h after inhalation of volatile anesthetics during mechanical ventilation. Although the mechanism cannot be determined from this study, TNF-α and IL-1β are the most important cytokines secreted from alveolar macrophages. Gene expression of TNF-α is reported to be rapid; for example, administration of lipopolysaccharide causes a marked increase in expression of the TNF-α gene within 30 min. Even the inhalation of 100% oxygen increases the gene expression of TNF-α within 3 h.[20] Consistent with these observations, the production of TNF-α cytokines is much faster in IL-1β.[21,22]

We observed significant increases in gene expression for IL-1β and IFN-γ, both of which are potent proinflammatory cytokines. Although we observed significant increases in gene expression for these cytokines, the concentrations did not increase in alveolar lavage fluid. Obviously, the increases in gene expression do not necessarily mean that the protein increases. However, the most obvious reason for discrepancy is that the study was insufficiently long. For example, protein expression of TNF-α was not detected until 24 h after exposure to 100% oxygen.[20]

Table 4. Cardiorespiratory Responses and Body Temperature with Various Volatile Anesthetics

Variable	Time	Control	Halothane	Enflurane	Isoflurane	Sevoflurane
MAP (mmHg)	Initial	101 ± 9	81 ± 7	86 ± 9	83 ± 9	86 ± 10
	Final	102 ± 10	86 ± 7*	89 ± 7*	88 ± 8*	89 ± 9*
HR (bpm)	Initial	423 ± 23	396 ± 19	391 ± 20	396 ± 14	393 ± 20
	Final	425 ± 25	403 ± 18*	401 ± 19*	402 ± 12*	403 ± 21*
T_{core} (°C)	Initial	37.1 ± 0.3	37.0 ± 0.3	37.1 ± 0.2	37.1 ± 0.3	37.0 ± 0.3
	Final	36.9 ± 0.3	36.9 ± 0.3	37.1 ± 0.3	37.0 ± 0.3	37.1 ± 0.2
*p*H	Initial	7.42 ± 0.04	7.44 ± 0.04	7.42 ± 0.04	7.43 ± 0.02	7.43 ± 0.03
	Final	7.42 ± 0.02	7.41 ± 0.03	7.42 ± 0.04	7.41 ± 0.03	7.42 ± 0.02
RR (rate/min)	Initial	29 ± 2	28 ± 2	30 ± 2	29 ± 2	30 ± 3
	Final	29 ± 2	28 ± 2	29 ± 2	29 ± 1	30 ± 2
Pa_{CO_2} (mmHg)	Initial	38.1 ± 2.1	37.6 ± 1.5	37.4 ± 1.3	37.2 ± 2.0	37.5 ± 1.5
	Final	37.8 ± 1.4	38.2 ± 1.2	37.4 ± 1.9	37.3 ± 1.7	37.2 ± 1.7
Pa_{O_2} (mmHg)	Initial	96.5 ± 7.7	97.1 ± 8.5	97.6 ± 7.4	99.6 ± 8.8	98.0 ± 8.0
	Final	98.1 ± 5.7	96.6 ± 6.1	97.3 ± 8.6	99.7 ± 8.1	100.3 ± 8.6

T_{core} = rectal temperature; RR = respiratory rate.

Values are mean ± SD. Initial values identify the beginning of the study; final values are those obtained 2 h later. There were no statistically significant differences from initial values.

* Significant difference from the control group.

Similarly, alveolar macrophages secreted TNF-α and IL-1β 4 and 8 h after lipopolysaccharide stimulation, respectively.[21,22] An additional possibility, however, is that cytokine production may be suppressed by volatile anesthetics. For example, Voisin *et al.*[23] and Hammer and Rannels[24] reported that exposure of alveolar macrophages to volatile anesthetics decreases protein production and intracellular ATP.

In this experiment, each volatile anesthetic produced a different pattern of gene expression and protein production. Many studies have shown suppression of neutrophil function with halothane and enflurane.[25-28] In contrast, isoflurane has only a minimal effect on the neutrophil function.[25,27] Mitsuhata *et al.*[29] reported that production of TNF-α and IL-1β is inhibited by enflurane, isoflurane, and sevoflurane. Sevoflurane was most effective at inhibiting cytokine release. This finding is consistent with our findings. To our knowledge, however, no studies have been published concerning the effects of various volatile anesthetics on gene expression or production of proinflammatory cytokines. Additional studies clearly are needed to elucidate the effects of various volatile anesthetics on alveolar macrophage function, and to determine why they provoke different responses. Our findings indicate that transcriptional regulation of cytokines is an important feature 2 h after the inhalation of volatile anesthetics under mechanical ventilation.

We could not detect gene expression and protein for

Table 5. Alveolar Cells and Pulmonary Histopathologic Changes with Various Volatile Anesthetics

	Mechanical Ventilation	Halothane (2 MAC)	Enflurane (2 MAC)	Isoflurane (2 MAC)	Sevoflurane (2 MAC)
Recovery rate (%)	84 ± 3	83 ± 3	83 ± 3	86 ± 2	84 ± 4
Cell concentration ($\times 10^5$/cm^3)	1.6 ± 0.3	1.5 ± 0.3	1.5 ± 0.3	1.6 ± 0.3	1.6 ± 0.3
Total cell number ($\times 10^6$)	6.7 ± 1.2	6.3 ± 1.1	6.2 ± 1.5	6.8 ± 1.3	6.5 ± 1.0
Macrophage (%)	96.7 ± 1.4	96.2 ± 1.8	96.4 ± 1.2	95.9 ± 1.4	97.0 ± 1.7
Lymphocyte (%)	1.5 ± 1.1	1.8 ± 0.8	1.8 ± 0.8	2.2 ± 0.8	1.9 ± 1.2
Neutrophils (%)	1.8 ± 0.8	2.0 ± 1.1	1.8 ± 0.8	1.9 ± 0.9	1.6 ± 1.5
Aggregation (%)	1.6 ± 1.7	2.6 ± 2.1	1.7 ± 1.5	2.3 ± 1.7	1.6 ± 1.2
Viability (%)	97.9 ± 1.1	97.3 ± 1.7	97.2 ± 1.6	97.2 ± 1.9	98.3 ± 1.5
Histology (0–4)	0 (0–1)	0 (0–0)	0 (0–1)	0 (0–0)	0 (0–0)
Wet/dry ratio	4.7 ± 0.4	4.8 ± 0.4	4.9 ± 0.3	4.8 ± 0.2	4.8 ± 0.3

Data are mean ± SD, except for histologic score, which is expressed in terms of median (range). There were no statistically significant differences among the groups.

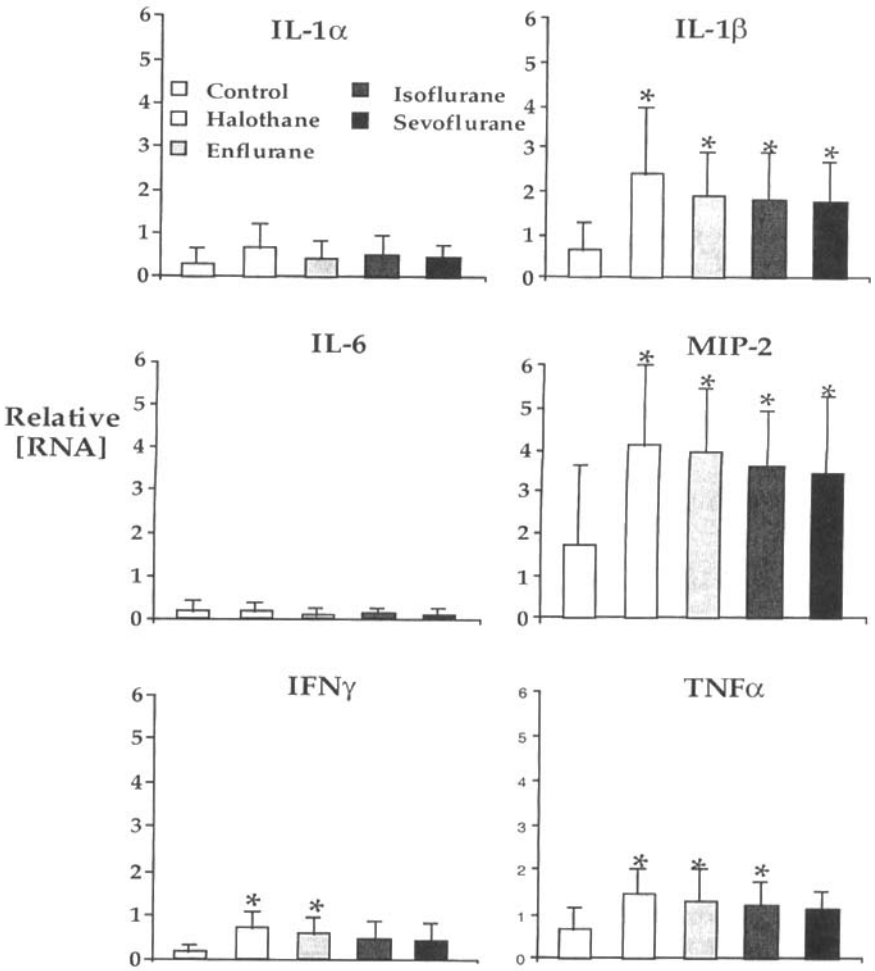

Fig. 3. Changes in expression of proinflammatory cytokines during mechanical ventilation without and with volatile anesthetics. Values are expressed and presented as the ratio of cytokine mRNA to β-actin mRNA and are presented as mean ± SD. Asterisks indicate statistically significant differences ($P < 0.05$) from the control group (mechanical ventilation without volatile anesthetics).

IL-1α and IL-6. Why this should be so remains unclear. IL-1α accounts for more than 90% of the IL-1 mRNA.[8] The failure of IL-1α detection may be attributed to the fact that the function of IL-1α is mainly intracellular and that IL-1α may be released by cellular damage.[30] Xing *et al.*[31] reported that expression of the gene for IL-6 was even more difficult to induce than of IL-1α, even with lipopolysaccharide. In contrast, von Bethmann *et al.*[32] reported that the gene expression of TNF-α and IL-6 were similar in positive- and negative-pressure ventilation on isolated mouse lungs. This discrepancy may be explained by the experimental model. One possibility for the failure of IL-6 detection may be the enzyme-linked immunoassay kit used in this study, because the minimum detection level (31 pg/ml) was much higher than for the other cytokines.

We did not separate the macrophages from other cells, such as lymphocyte and neutrophils, to avoid any artificial influence of the sorting process on cytokine expression. For example, even adherence to plastic test tubes for separation of alveolar macrophage can induce gene expression of cytokines.[33] Thus, we cannot draw a definite conclusion regarding the cell source for the observed increase in gene transcription. However, macrophages are the likely source, because there is considerable evidence that they produce most proinflammatory cytokines.[14] Furthermore, at least 95% of the harvested cells were macrophages.

In conclusion, we found that inhalation of volatile anesthetics under mechanical ventilation augments gene expression of proinflammatory cytokines. Furthermore, some of cytokine concentration increased after the experiment. Our results indicate that transcriptional regulation of cytokines is an important feature 2 h after

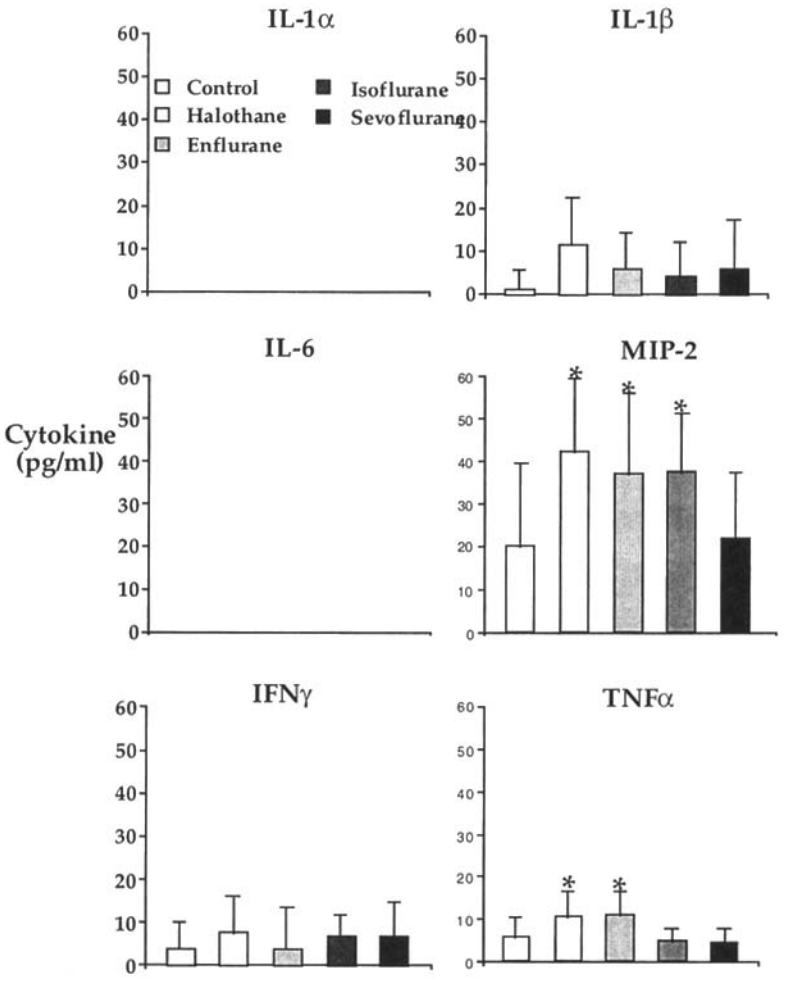

Fig. 4. Changes in absolute pulmonary lavage concentration of proinflammatory cytokines during mechanical ventilation without and with various volatile anesthetics. Data are expressed as mean ± SD. Asterisks indicate statistically significant differences ($P < 0.05$) from the control group (mechanical ventilation without volatile anesthetics).

inhalation of volatile anesthetics under mechanical ventilation.

References

1. Kotani N, Lin CY, Wang JS, Gurley JM, Tolin FP, Michelassi F, Lin HS, Sandberg WS, Roizen MF: Loss of alveolar macrophages during anesthesia and operation in humans. Anesth Analg 1995; 81:1255-62

2. Kotani N, Hashimoto H, Sessler DI, Kikuchi A, Suzuki A, Takahashi S, Muraoka M, Matsuki A: Intraoperative modulation of alveolar macrophage functions during isoflurane and propofol anesthesia. ANESTHESIOLOGY 1998; 89:1125-32

3. Vandenburgh HH: Mechanical forces and their second messengers in stimulating cell growth in vitro. Am J Physiol 1992; 262:R350-5

4. Grigg J, Kukielka GL, Berens KL, Dreyer WJ, Entman ML, Smith CW: Induction of intercellular adhesion molecule-1 by lipopolysaccharide in canine alveolar macrophages. Am J Respir Cell Mol Biol 1994; 11:304-11

5. Chollet Martin S, Jourdain B, Gibert C, Elbim C, Chastre J, Gougerot Pocidalo MA: Interactions between neutrophils and cytokines in blood and alveolar spaces during ARDS. Am J Respir Crit Care Med 1996; 154:594-601

6. Haga Y, Beppu T, Doi K, Nozawa F, Mugita N, Ikei S, Ogawa M: Systemic inflammatory response syndrome and organ dysfunction following gastrointestinal surgery. Crit Care Med 1997; 25:1994-2000

7. Parker JC, Hernandez LA, Peevy KJ: Mechanisms of ventilator-induced lung injury. Crit Care Med 1993; 21:131-43

8. Kelley J: Cytokines of the lung. Am Rev Respir Dis 1990; 141: 765-88

9. Elias JA, Freundlich B, Kern JA, Rosenbloom J: Cytokine networks in the regulation of inflammation and fibrosis in the lung. Chest 1990; 97:1439-45

10. Nishina K, Mikawa K, Takao Y, Maekawa N, Shiga M, Obara H: ONO-5046, an elastase inhibitor, attenuates endotoxin-induced acute lung injury in rabbits. Anesth Analg 1997; 84:1097-103

11. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162:156-9

12. Kolls JK, Beck JM, Nelson S, Summer WR, Shellito J: Alveolar macrophage release of tumor necrosis factor during murine *Pneumocystis carinii* pneumonia. Am J Respir Cell Mol Biol 1993; 8:370-6

13. Maeda A, Hiyama K, Yamakido H, Ishioka S, Yamakido M: Increased expression of platelet-derived growth factor A and insulin-like growth factor-I in BAL cells during the development of bleomycin-induced pulmonary fibrosis in mice. Chest 1996; 109:780–6

14. Takata M, Abe J, Tanaka H, Kitano Y, Doi S, Kohsaka T, Miyasaka K: Intraalveolar expression of tumor necrosis factor-alpha gene during conventional and high-frequency ventilation. Am J Respir Crit Care Med 1997; 156:272–9

15. Gupta S, Feng L, Yoshimura T, Redick J, Fu SM, Rose CE Jr: Intra-alveolar macrophage-inflammatory peptide 2 induces rapid neutrophil localization in the lung. Am J Respir Cell Mol Biol 1996; 15:656–63

16. Xing Z, Jordana M, Kirpalani H, Driscoll KE, Schall TJ, Gauldie J: Cytokine expression by neutrophils and macrophages in vivo: Endotoxin induces tumor necrosis factor-alpha, macrophage inflammatory protein-2, interleukin-1 beta, and interleukin-6 but not RANTES or transforming growth factor-beta 1 mRNA expression in acute lung inflammation. Am J Respir Cell Mol Biol 1994; 10: 148–53

17. Ulich TR, Watson LR, Yin SM, Guo KZ, Wang P, Thang H, del Castillo J: The intratracheal administration of endotoxin and cytokines. I. Characterization of LPS-induced IL-1 and TNF mRNA expression and the LPS-, IL-1-, and TNF-induced inflammatory infiltrate. Am J Pathol 1991; 138:1485–96

18. Farone A, Huang S, Paulauskis J, Kobzik L: Airway neutrophilia and chemokine mRNA expression in sulfur dioxide-induced bronchitis. Am J Respir Cell Mol Biol 1995; 12:345–50

19. Driscoll KE, Simpson L, Carter J, Hassenbein D, Leikauf GD: Ozone inhalation stimulates expression of a neutrophil chemotactic protein, macrophage inflammatory protein 2. Toxicol Appl Pharmacol 1993; 119:306–9

20. Horinouchi H, Wang CC, Shepherd KE, Jones R: TNF alpha gene and protein expression in alveolar macrophages in acute and chronic hyperoxia-induced lung injury. Am J Respir Cell Mol Biol 1996; 14:548–55

21. Ulich TR, Howard SC, Remick DG, Wittwer A, Yi ES, Yin S, Guo K, Welply JK, Williams JH: Intratracheal administration of endotoxin and cytokines. VI. Antiserum to CINC inhibits acute inflammation. Am J Physiol 1995; 268:L245–50

22. Ulich TR, Guo K, Yin S, del Castillo J, Yi ES, Thompson RC, Eisenberg SP: Endotoxin-induced cytokine gene expression in vivo. IV. Expression of interleukin-1 alpha/beta and interleukin-1 receptor antagonist mRNA during endotoxemia and during endotoxin-initiated local acute inflammation. Am J Pathol 1992; 141:61–8

23. Voisin C, Scherpereel PA, Aerts C, Lepot D: In vitro toxicity of halogenated anaesthetics on guineapig alveolar macrophages, surviving in gas phase. Br J Anaesth 1984; 56:415–20

24. Hammer JAd, Rannels DE: Effects of halothane on protein synthesis and degradation in rabbit pulmonary macrophages. Am Rev Respir Dis 1981; 124:50–5

25. Welch WD: Effect of enflurane, isoflurane, and nitrous oxide on the microbicidal activity of human polymorphonuclear leukocytes. ANESTHESIOLOGY 1984; 61:188–92

26. Lieners C, Redl H, Schlag G, Hammerschmidt DE: Inhibition by halothane, but not by isoflurane, of oxidative response to opsonized zymosan in whole blood. Inflammation 1989; 13:621–30

27. Nakagawara M, Takeshige K, Takamatsu J, Takahashi S, Yoshitake J, Minakami S: Inhibition of superoxide production and Ca^{2+} mobilization in human neutrophils by halothane, enflurane, and isoflurane. ANESTHESIOLOGY 1986; 64:4–12

28. Frohlich D, Rothe G, Schwall B, Schmid P, Schmitz G, Taeger K, Hobbhahn J: Effects of volatile anaesthetics on human neutrophil oxidative response to the bacterial peptide FMLP. Br J Anaesth 1997; 78:718–23

29. Mitsuhata H, Shimizu R, Yokoyama MM: Suppressive effects of volatile anesthetics on cytokine release in human peripheral blood mononuclear cells. Int J Immunopharmacol 1995; 17:529–34

30. Hack CE, Aarden LA, Thijs LG: Role of cytokines in sepsis. Adv Immunol 1997; 66:101–95

31. Xing Z, Jordana M, Gauldie J: IL-1 beta and IL-6 gene expression in alveolar macrophages: Modulation by extracellular matrices. Am J Physiol 1992; 262:L600–5

32. von Bethmann AN, Brasch F, Nusing R, Vogt K, Volk HD, Muller KM, Wendel A, Uhlig S: Hyperventilation induces release of cytokines from perfused mouse lung. Am J Respir Crit Care Med 1998; 157:263–72

33. Northemann W, Braciak TA, Hattori M, Lee F, Fey GH: Structure of the rat interleukin 6 gene and its expression in macrophage-derived cells. J Biol Chem 1989; 264:16072–82