Intraoperative Modulation of Alveolar Macrophage Function during Isoflurane and Propofol Anesthesia

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Background: Alveolar macrophages are a critical part of the defense against pulmonary infection. Thus the authors determined time-dependent changes in alveolar macrophage functions in patients having surgery who were anesthetized with isoflurane or propofol.

Methods: Patients anesthetized with propofol (n = 30) or isoflurane (n = 30) during orthopedic surgery were studied. Alveolar macrophages were harvested by bronchoalveolar lavage immediately, and 2, 4, and 6 h after induction anesthesia and at the end of surgery. The fraction of aggregated and nonviable macrophages was determined. Then phagocytosis was measured by ingestion of opsonized and unopsonized particles. Finally, microbicidal activity was determined as the ability of the macrophages to kill *Listeria monocytogenes* directly.

Results: Demographic and morphometric characteristics of the patients given propofol and isoflurane were similar, as were their levels of pulmonary function and hemodynamic responses. The fraction of alveolar macrophages ingesting opsonized and unopsonized particles, and the number of particles ingested, decreased significantly over time, with the decrease slightly but significantly greater during isoflurane anesthesia. Microbicidal function decreased progressively during anesthesia and surgery, with the decrease almost twice as great during isoflurane compared with propofol anesthesia. The fraction of aggregated macrophages and recovered neutrophils increased over time in the patients given each anesthetic.

Conclusions: Pulmonary immunologic function changed progressively during anesthesia and surgery. The data from this study suggest that pulmonary defenses are modulated by the type of anesthesia and by the duration of anesthesia and surgery. (Key words: Aggregation; bronchoalveolar lavage; immune function; infection; microbicidal activity; phagocytosis; pulmonary function; surgery.)

FACTORS contributing to postoperative pulmonary infections have been evaluated.[1-3] However, few studies have demonstrated time-dependent pulmonary changes during general anesthesia. In a previous preliminary investigation,[4] we quantified the effects of surgery and anesthesia on alveolar macrophages and observed increased aggregation and decreased viability with increased duration of anesthesia and surgery. However, we did not evaluate time-dependent changes in several fundamental functions of alveolar macrophages, such as phagocytosis and microbicidal activity.

We were also interested in whether the general anesthesia itself might have some role in the occurrence of such events. Because alveolar macrophages mainly react to inhaled foreign substances, volatile and intravenous anesthetics may change immune functions of alveolar macrophages differently. Our aim was to evaluate the time-dependent changes in the alveolar macrophage functions, including phagocytosis, microbicidal activity, aggregation, and viability during surgery with isoflurane or propofol anesthesia.

Methods

The protocol of this study was approved by the Institutional Review Board of the University of Hirosaki, and

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written informed consent was obtained from all partici-pants. We studied 60 patients whose orthopedic surgery was expected to exceed 6 h.

Patients with one or more of the following conditions were excluded: abdominal and thoracic surgery; a his-tory of current smoking; current use of steroid or non-steroidal anti-inflammatory medications; American Soci-ety of Anesthesiologists physical status II or higher; chronic obstructive or restrictive pulmonary disease; pulmonary or other infection or abnormal results of chest radiograph; neoplastic disease; forced vital capac-ity and forced expiratory volume in 1 s < 80% and 70% of the predicted value, respectively; or a body mass index exceeding 30%.

Protocol

Patients were randomly assigned to anesthetic mainte-nance with either propofol (n = 30) or isoflurane (n = 30). Patients in each group were supplemented with intravenous fentanyl and vecuronium. During anesthe-sia, all the patients were mechanically ventilated with 30% oxygen and 70% nitrogen to maintain the carbon dioxide tension in arterial blood between 35 and 45 mmHg.

Bronchoalveolar lavages were performed immediately after induction, at 2, 4, and 6 h after induction of anes-thesia, and at the end of the surgery. Before each bron-choalveolar lavage, arterial blood was sampled for gas analysis. We used a slight modification of our previously described method for this study.[4] A bronchovideoscope (BF type P200, CV200, CLV-U20D; Olympus, Tokyo, Ja-pan) was introduced through the endotracheal tube while mechanical ventilation was maintained. The tip of the bronchoscope was wedged into the left or right segment of the lower or middle lobe of the lungs. This segment was then lavaged *via* the suction port after instilling 20 ml sterile balanced saline solution contain-ing 125 mM NaCl, 6 mM KCl, 10 mM dextrose, and 20 mM HEPES titrated with NaOH to a *p*H of 7.4. Then the pulmonary lavage fluid was gently aspirated. This proce-dure was repeated five times, with 100 ml solution instilled in total. A different randomly chosen segment was lavaged at each time point; the same investigator performed all the bronchoalveolar lavages.

Measurements

Alveolar macrophages were isolated from the fluid recovered by bronchoalveolar lavage, and the cell type, viability, and aggregation were determined using a slight modification of our previously described tech-nique.[4] Briefly, after straining through a single layer of loose cotton gauze to remove mucus, we pooled the aspirated fluid in a sterile siliconized container and counted the number of alveolar macrophages with a hemocytometer. Cells were considered viable when they could not be stained with trypan blue (0.2%); viability was estimated from a count of 200 cells. Cells were also stained with Wright-Giemsa stain (American Hospital Supply Corp., McGraw Park, IL), and the cell type and aggregation were determined on the stained slides by light microscopic examination. Erythrocytes and ciliated cells, constituting <5% of harvested cells, were not counted. Aggregation of the alveolar macro-phages was expressed as a percentage of nuclei in aggregated cells per 500 nuclei counted on two or three slide preparations. Then lavage fluid was divided into two equal volumes for assay determination of phagocytosis and bactericidal activity.

Alveolar macrophages were separated from bronchoal-veolar lavage fluid by centrifugation at 200g for 10 min. After the supernatant was decanted, alveolar macro-phages were resuspended at a concentration of 0.5×10^6 cells/ml in a balanced saline solution containing 0.3 mM $CaCl_2$ and 1 mM $MgCl_2$. We evaluated phagocytosis by the ability of alveolar macrophages to ingest both the unopsonized and opsonized fluorescent particles (1 μm diameter) with a modification of the method described by Kobzik *et al.*[5] Excitation and emission wave lengths of the particles were 488 and 530 nm, respectively. The particles were coated with bovine serum albumin by incubation in a 1-mg/ml solution at 37°C for 1 h. These unopsonized particles were used to measure opsonin-independent phagocytosis. To make opsonized particles, rabbit immunoglobulin G anti-bovine serum albumin (10 mg/ml) was incubated with the albumin-coated particles at 37°C for 30 min. Both unopsonized and opsonized particles were washed three times with the balanced saline solution and stored at −20°C until use.

Resuspended alveolar macrophages were incubated as suspensions at 37°C in 20-ml sterile centrifuge tubes on a shaking platform (60 cycles/min). The unopsonized and the opsonized particles were added to the separate centrifuge tubes, each containing a sample of the cell suspension; the particle-to-cell ratios were 15:1, and the total assay volume was 1 ml. The tubes were incubated for 15 min, and the phagocytosis was stopped by the addition of 2 ml ice-cold balanced saline solution. After centrifugation for 2 min at 200g, the cell pellet was resuspended in 0.1 ml ice-cold balanced saline solution. This cell suspension was placed on a glass slide, fixed

with 4% paraformaldehyde, and washed and stained with hematoxylin. We counted 400 alveolar macrophages with a fluorescence microscope and recorded the fraction that ingested at least one particle and also the number of fluorescent particles per positive phagocytic alveolar macrophage. In a preliminary study, we confirmed that neutrophils had no effect on macrophage phagocytosis.

The bactericidal ability of the alveolar macrophages was determined by their ability to kill *Listeria monocytogenes* using a modification of a previously described method.[6] *Listeria* were suspended at a concentration of 1×10^9 colony-forming U/ml in RPMI-1640 and stored at $-80°C$ until use. A preliminary study indicated that normal human serum was required to help kill *Listeria*. Consequently, human serum was pooled and stored at $-80°C$; the same batch of serum was used for the entire study. Alveolar macrophages were separated as in the phagocytosis assay at 2-h intervals and at the end of surgery. We resuspended each set of alveolar cells at a concentration of 0.5×10^6 cells/ml in RPMI-1640 and plated them in 24-well dishes. The cells were incubated for 30 min before nonadherent cells were removed with three washes with warm RPMI-1640. The adherent cells were finally resuspended in 0.5 ml RPMI containing 10% normal human serum. More than 98% of adherent cells were macrophages.

The bacteria were resuspended in the same medium at a concentration of 2×10^6 colony-forming U/ml. Resuspended aliquots of *Listeria* (0.5 ml) were mixed and incubated for 30 min and 120 min in 5% carbon dioxide and air. Control tubes contained the RPMI, human serum, and *Listeria*. The pellets of alveolar macrophages were lysed by adding 10 ml sterilized distilled water and

Table 1. Morphometric and Demographic Characteristics, Anesthetic and Surgical Management, and Pulmonary Responses

	Propofol	Isoflurane
Age (yr)	41 ± 9	42 ± 11
Gender (M/F)	17/13	16/14
Weight (kg)	62 ± 10	65 ± 7
Height (cm)	166 ± 10	169 ± 7
ASA Physical Status (1/2)	16/14	15/15
FVC (% predicted)	97 ± 11	97 ± 13
FEV (% FVC)	84 ± 7	82 ± 8
Duration of anesthesia (h)	8.6 ± 1.1	8.9 ± 1.3
Total fentanyl (mg)	0.7 ± 0.2	0.6 ± 0.2

FVC = forced vital capacity; FEV = forced expiratory volume.
Results are mean ± SD. There were no statistically significant differences between the groups.

Table 2. Operative Procedures

	Propofol	Isoflurane
Posterolateral fusion	8	8
Rotational acetabular osteotomy	7	6
Anterior rotational osteotomy	6	9
Vascularized fibular graft for pseudoarthorsis	6	5
Partial toe transfer	3	2

Operative procedures were similar in each group.

vortexing for 30 s to release bacteria. The viable fraction of *Listeria* bacteria was determined by plating serial 10-fold dilutions (10–1,000 times) on agar plates. The number of colonies of *Listeria* was counted after 48 h on one of the plates.

The rate at which alveolar macrophages killed *Listeria* was calculated by dividing the fraction of the initial inoculum of *Listeria* killed by the fraction of the initial inoculum surviving in the control (cell-free) tubes. This method thus corrected for bacterial survival over time and the bactericidal effect of normal human serum. All immune function tests were performed by a single investigator who was blinded to the anesthetic management and elapsed time from induction of anesthesia.

Data Analysis

The time immediately after induction of anesthesia was designated elapsed time zero. Time-dependent intragroup data were evaluated using repeated-measures analysis of variance and Dunnett's tests for comparison with control values. Probability values < 0.05 were considered significant. Differences between propofol and isoflurane at each time point were evaluated using two-tailed, unpaired t tests or chi-squared tests, as appropriate. Our nominal P value was 0.05. But because we compared values in the two groups at five time points, we used a Bonferonni correction. A $P < 0.01$ was thus considered significant. Data are expressed as mean ± SD.

Results

Demographic and morphometric characteristics were similar in the two groups (table 1), as were the operative procedures (table 2). There were no significant differences in intraoperative measurements between the two groups (table 3), and patients did not experience postoperative complications. There were no significant differences in the cell-recovery rates and the concentration

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Table 3. Potential Confounding Factors in Two Groups

		Elapsed Time				
		0 h	2 h	4 h	6 h	End
MAP (mmHg)	Prop	75 ± 7	84 ± 10*	83 ± 10*	85 ± 8*	84 ± 9*
	Iso	75 ± 5	80 ± 16*	83 ± 10*	85 ± 9*	83 ± 7*
HR (bpm)	Prop	74 ± 9	78 ± 13*	77 ± 12*	77 ± 11*	79 ± 10*
	Iso	75 ± 11	81 ± 14*	82 ± 11*	82 ± 11*	82 ± 10*
Arterial pH	Prop	7.41 ± 0.03	7.39 ± 0.03*	7.37 ± 0.03*	7.34 ± 0.04*	7.31 ± 0.03*
	Iso	7.42 ± 0.03	7.40 ± 0.03*	7.37 ± 0.04*	7.33 ± 0.03*	7.31 ± 0.04*
Arterial PO_2 (mmHg)	Prop	128 ± 22	128 ± 21	134 ± 17	132 ± 20	126 ± 18
	Iso	126 ± 25	125 ± 23	125 ± 22	124 ± 20	120 ± 20
Arterial PCO_2 (mmHg).	Prop	39 ± 3	39 ± 3	39 ± 2	39 ± 2	39 ± 2
	Iso	40 ± 3	40 ± 2	40 ± 2	40 ± 2	40 ± 2
T_{core} (°C)	Prop	36.6 ± 0.3	36.3 ± 0.4*	36.2 ± 0.5*	36.3 ± 0.5*	35.9 ± 0.6*
	Iso	36.7 ± 0.3	36.3 ± 0.4*	36.4 ± 0.5*	36.3 ± 0.5*	36.0 ± 0.6*

T_{core} = core temperature; Prop = propofol; Iso = isoflurane.

Results are mean ± SD.

* Statistically significant differences from elapsed time zero (just after induction of anesthesia). There were no significant differences between the patients given propofol and isoflurane.

as a function of time within groups or between groups. However, the percentage of neutrophils increased significantly over time, whereas the fraction of macrophages decreased significantly. The percentage of lymphocytes did not change (table 4).

The fraction of alveolar macrophages ingesting opsonized and unopsonized particles decreased significantly 4 h after induction of anesthesia, and values in each group differed significantly from control values. The decrease in both unopsonized and opsonized phagocytosis reached 30–40% by the end of surgery. After 4 h of anesthesia, the between-group differences were small (5–10%) but significantly greater during isoflurane than propofol anesthesia (fig. 1).

The number of opsonized and unopsonized particles ingested in macrophages (ingesting at least one particle)

also decreased significantly in both groups starting 4 h after induction of anesthesia. The decrease in the number of ingested opsonized particles was slightly (0.5 to 1 bead/cell) but significantly greater with isoflurane than propofol anesthesia starting 6 h after induction (fig. 2).

Similar to phagocytosis, microbicidal activity evaluated by the killing rate of *L. monocytogenes* at both 30 and 120 min after incubation decreased as the duration of anesthesia increased. By the end of surgery, microbicidal activity decreased 30–35% in the patients given isoflurane, whereas it decreased 15–20% in those given propofol; both the difference over time and the differences between the groups were significant (fig. 3).

The fraction of nonviable recovered alveolar macrophages did not change significantly over time. The fraction of aggregated macrophages doubled during the

Table 4. Cell Recovery from Bronchoalveolar Lavage

		0 h	2 h	4 h	6 h	End
Recovery rate (%)	Propofol	54 ± 7	55 ± 8	55 ± 8	54 ± 7	54. ± 8
	Isoflurane	52 ± 8	52 ± 8	52 ± 8	52 ± 9	51 ± 9
Cells (× 10^5/cm³)	Propofol	1.5 ± 0.5	1.5 ± 0.4	1.5 ± 0.4	1.6 ± 0.5	1.6 ± 4
	Isoflurane	1.3 ± 0.5	1.2 ± 0.5	1.3 ± 0.5	1.3 ± 0.5	1.3 ± 0.5
Macrophages (%)	Propofol	91.1 ± 3.5	91.0 ± 3.1	88.8 ± 3.9*	87.5 ± 5.3*	84.3 ± 6.9*
	Isoflurane	91.2 ± 3.0	91.3 ± 3.4	88.7 ± 4.3*	85.6 ± 4.3*	82.1 ± 6.0*
Lymphocytes (%)	Propofol	7.6 ± 2.8	7.5 ± 2.8	7.7 ± 3.6	7.3 ± 4.2	7.7 ± 4.6
	Isoflurane	7.3 ± 2.8	7.2 ± 3.0	7.4 ± 3.8	7.5 ± 3.8	7.3 ± 4.6
Neutrophils (%)	Propofol	1.3 ± 1.2	1.5 ± 1.2	3.5 ± 1.6*	5.3 ± 2.4*	8.0 ± 4.0*
	Isoflurane	1.5 ± 1.5	1.5 ± 1.6	3.8 ± 2.3*	6.9 ± 3.1*	10.6 ± 4.2*

Data are mean ± SD.

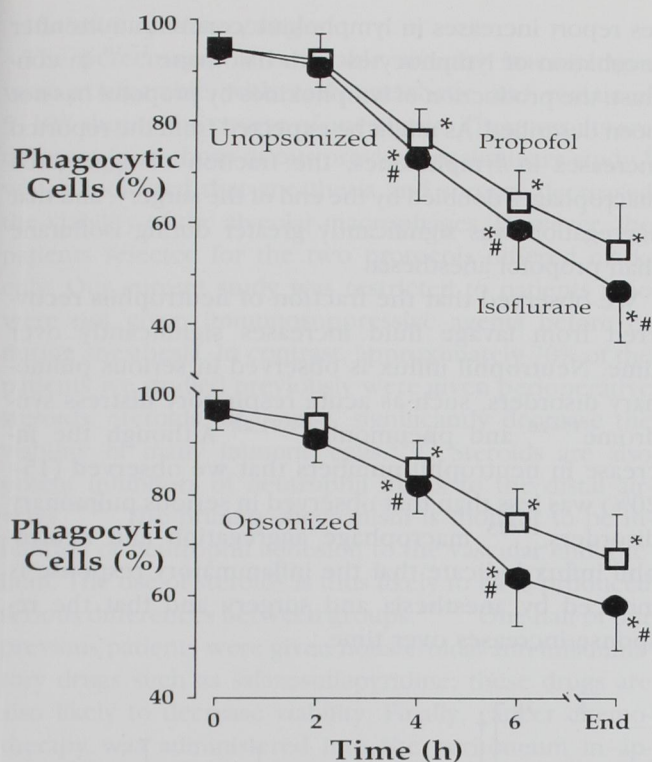* Statistically significant differences from control values.

Fig. 1. The fraction of alveolar macrophages ingesting opsonized and unopsonized particles in patients given propofol (*squares*) and isoflurane (*circles*). *Significant differences from control values. #Significant differences between propofol and isoflurane. Data are expressed as mean ± SD.

study period in both groups, and the increase in aggregation was significantly greater in isoflurane anesthesia than propofol anesthesia starting 6 h after induction of anesthesia (fig. 4).

Discussion

Phagocytic and Microbicidal Activities

Two ligand-receptor mechanisms mediate phagocytosis. One facilitates ingestion of particles coated with immunoglobulin G or fragments of the third complement component (C3); this is called *opsonin-dependent phagocytosis*. A second phagocytic mechanism mediates ingestion of particles in the absence of immunoglobulin or complement, and it is called *opsonin-independent phagocytosis*.

An important finding in our study is that the intraoperative opsonin-dependent and opsonin-independent phagocytosis by alveolar macrophages decreased significantly over time. A similar reduction in phagocytosis of peripheral blood monocytes has been reported during

and after anesthesia and surgery.[7–9] These reductions presumably result in part from the direct anesthetic-induced inhibition of phagocytic and chemotactic activity in many immune cells, including polymorphonuclear neutrophils.[7,10,11] Consistent with the importance of anesthesia *per se*, several studies indicate that regional anesthesia negates the decrease in phagocytosis.[9,12] The mechanism by which isoflurane and propofol anesthesia cause these differences remains unclear.

Production of oxygen free-radicals is essential for monocyte–macrophage–neutrophil-related bactericidal activity. Therefore many previous studies have used oxidative ability to evaluate bactericidal activity. Instead we evaluated bactericidal ability, which may more closely represent *in vivo* bactericidal activity than previous studies did. Our most notable finding was a substantial time-dependent reduction in microbicidal activity. Further, isoflurane anesthesia decreased microbicidal activ-
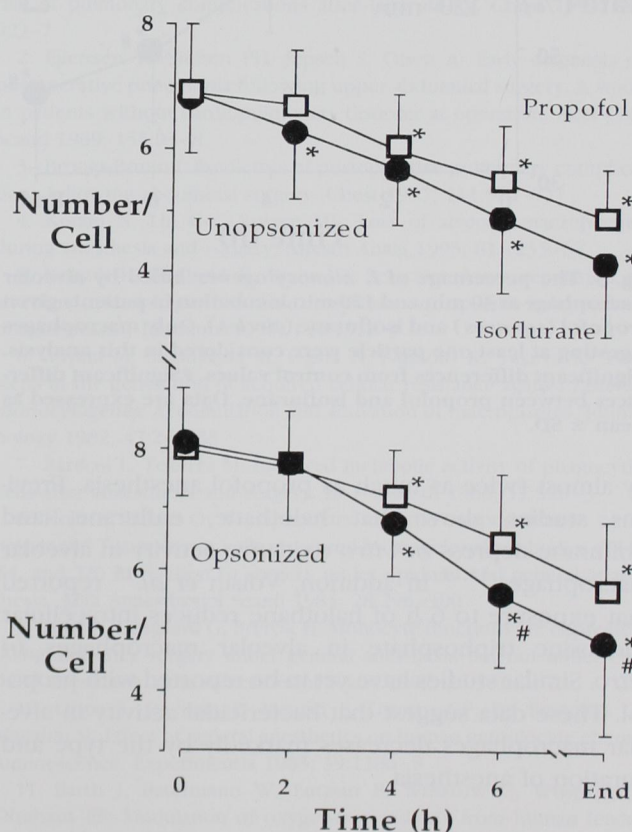

Fig. 2. The number of opsonized and unopsonized particles ingested by alveolar macrophages in patients given propofol (*squares*) and isoflurane (*circles*). Only macrophages ingesting at least one particle were considered in this analysis. *Significant differences from control values. #Significant differences between propofol and isoflurane. Data are expressed as mean ± SD.

Fig. 3. The percentage of *L. monocytogenes* killed by alveolar macrophage at 30 min and 120 min incubation in patients given propofol (*squares*) and isoflurane (*circles*). Only macrophages ingesting at least one particle were considered in this analysis. *Significant differences from control values. #Significant differences between propofol and isoflurane. Data are expressed as mean ± SD.
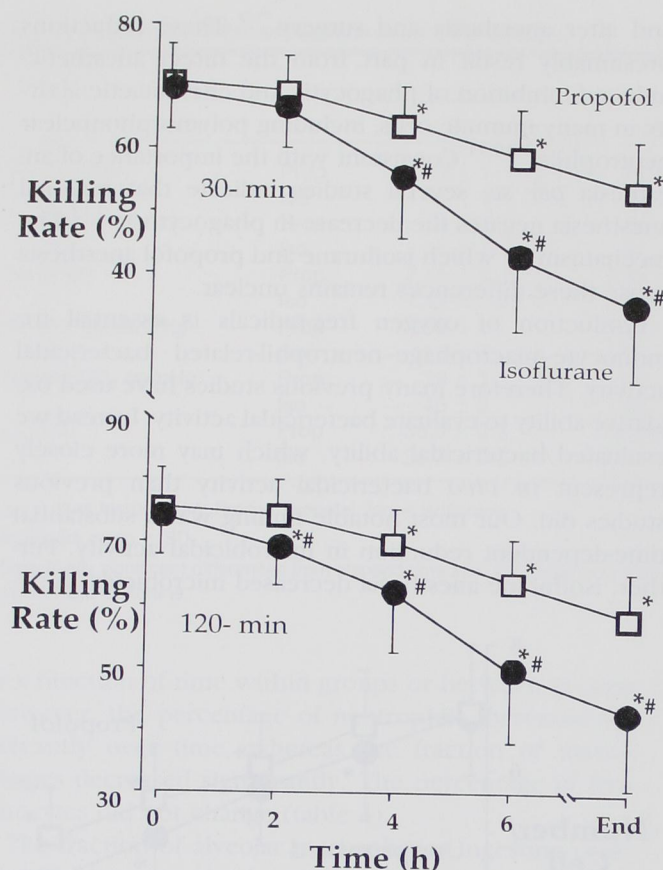
ity almost twice as much as propofol anesthesia. Previous studies show that halothane, enflurane, and isoflurane depress *in vitro* oxidative activity in alveolar macrophages.[13,14] In addition, Voisin *et al.*[15] reported that exposure to 6 h of halothane reduces intracellular adenosine triphosphate in alveolar macrophages *in vitro*. Similar studies have yet to be reported with propofol. These data suggest that bactericidal activity in alveolar macrophages decreases markedly by the type and duration of anesthesia.

Neutrophil Influx and Aggregation of Macrophages

Macrophage aggregation occurs in many inflammatory conditions. Aggregation is facilitated by the release of lymphokines such as macrophage-aggregating factors and interferon-gamma, and it depends on temperature and calcium and magnesium concentrations.[16] Two stud-

ies report increases in lymphokine concentrations after incubation of lymphocytes with halothane.[17,18] In contrast, the production of lymphokines by propofol has not been described. As might be expected from the reported increases in lymphokines, the fraction of aggregated macrophages doubled by the end of the surgery, and that aggregation was significantly greater during isoflurane than propofol anesthesia.

We observed that the fraction of neutrophils recovered from lavage fluid increases significantly over time. Neutrophil influx is observed in serious pulmonary disorders, such as acute respiratory distress syndrome[17-20] and pneumonia.[17,21,22] Although the increase in neutrophil numbers that we observed (15–20%) was less than that observed in serious pulmonary disorders,[17-23] macrophage aggregation and neutrophil influx indicate that the inflammatory response is induced by anesthesia and surgery and that the response increases over time.
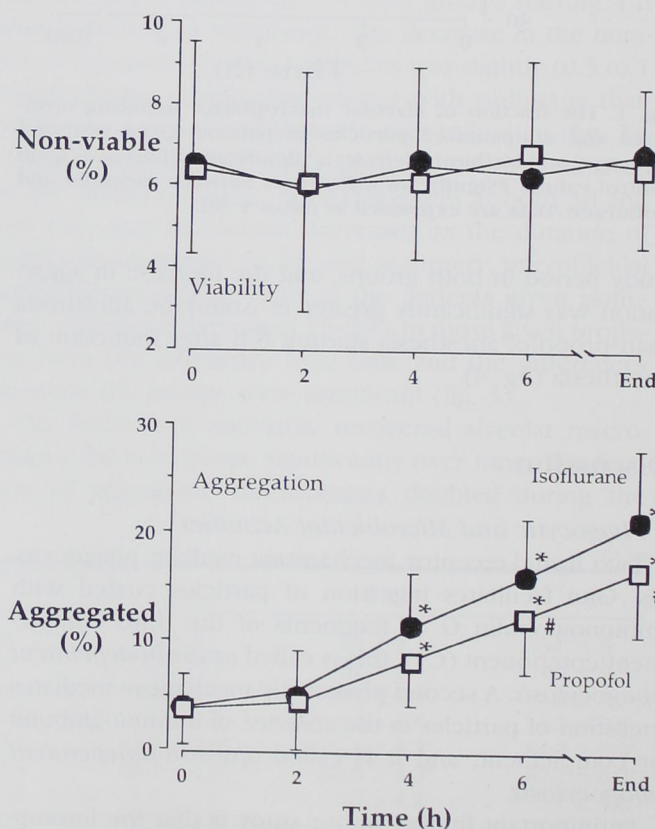

Fig. 4. The fraction of aggregated and nonviable alveolar macrophages in patients given propofol (*squares*) and isoflurane (*circles*). *Significant differences from control values. #Significant differences between propofol and isoflurane. Data are expressed as mean ± SD.

Viability of Macrophages

The percentages of nonviable alveolar macrophages were comparable with each anesthetic and remained 5-10% during the course of anesthesia. These results are different from those of our previous preliminary study,[4] which indicated that anesthesia and surgery decreased the viability of the alveolar macrophages. However, the patients selected for the two protocols differed markedly. Our current study was restricted to patients who were not given immunosuppressive agents before or during anesthesia. In contrast, approximately 70% of the patients we studied previously were given perioperative steroids. Steroids, of course, significantly decrease the viability of many immune cells.[24,25] Steroids are also potent inhibitors of neutrophil influx to the distal airways.[26,27] The primary mechanism is thought to be inhibition of neutrophil adhesion to the vascular endothelium. The use of steroids is thus likely to have produced serious differences between groups.[28-30] One half of our previous patients were given nonsteroidal anti-inflammatory drugs such as salazosulfapyridine; these drugs are also likely to decrease viability. Finally, cancer chemotherapy was administered into the peritoneum in approximately 15% of our previous patients during colon resections. Anti-cancer agents obviously kill alveolar macrophages.[31] Variance in our previous and current alveolar viability data thus likely results from important differences in the patients studied.

Study Limitations

All of our patients were anesthetized, undergoing surgery, and mechanically ventilated, so we cannot determine the independent contributions of each factor. However, it is likely that all contributed to the observed time-dependent alterations in pulmonary immune function. We similarly could not determine whether the observed differences between isoflurane and propofol might be a reasonable basis for choosing one agent or another.

Alveolar macrophages have important immunologic functions as effector and regulatory cells. Their regulatory functions include recognition of pulmonary insults and initiation of adequate inflammatory reactions *via* secretion of immunomodulators. The progressive neutrophil influx and macrophage aggregation observed in this study suggest that anesthesia and surgery provoke a progressive inflammatory reaction of alveolar macrophages. Given this situation, alveolar macrophages appear to function more as regulatory cells, with effector functions including phagocytosis and bactericidal activ-

ity being suppressed. Whether we observed an appropriate defense against pulmonary insult or a harmful inflammatory reaction remains to be determined.

In conclusion, pulmonary macrophage aggregation and neutrophil influx increased significantly over time, whereas phagocytic and microbicidal activity decreased. These changes were more pronounced during isoflurane than propofol anesthesia, suggesting that the immunologic functions of alveolar macrophages are modulated by the type of anesthesia and the duration of anesthesia and surgery.

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