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Supplemental Intraoperative Oxygen Augments Antimicrobial and Proinflammatory Responses of Alveolar Macrophages

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Background: **The first goal was to test the hypothesis that 100% inspired oxygen maintained for approximately 8 h intraoperatively is not associated with impaired pulmonary oxygenation. The authors also tested the hypothesis that intraoperative inhalation of 100% oxygen augments proinflammatory and antimicrobial responses of alveolar macrophages during anesthesia and surgery.**

Methods: **The authors studied patients administered 100% oxygen (n = 30) and 30% oxygen (n = 30) during propofol–fentanyl general anesthesia. Alveolar macrophages were harvested by bronchoalveolar lavage immediately, 2, 4, and 6 h after induction of anesthesia, and at the end of surgery.The authors measured "opsonized" and "unopsonized" phagocytosis and microbicidal activity. RNA was extracted from harvested cells and cDNA was synthesized. The expression of interleukin(IL)–1β, IL-6, IL-8, interferon-γ (IFN-γ) and tumor necrosis factor α (TNF-α) was measured by semiquantitative polymerase chain reaction.**

Results: **Gene expression of all proinflammatory cytokines except IL-6 increased fourfold to 20-fold over time in both groups. However, expression of TNF-α and IL-8, IFN-γ, and IL-6 and IL-1β was 2–20 times greater in patients administered 100% than in those administered 30% oxygen. Unopsonized and opsonized phagocytosis and microbicidal activity decreased progressively, with the decreases being nearly twice as great during inhalation of 30% oxygen versus 100% oxygen.**

Conclusion: **Inhalation of 100% oxygen improved intraoperative decreases in phagocytic and microbicidal activity possibly because expression of proinflammatory cytokines was augmented. These data therefore suggest that intraoperative inhalation of 100% oxygen augments antimicrobial and proinflammatory responses in alveolar macrophages during anesthesia and surgery. (Key words: Aggregation; anesthesia; cytokines; gene expression; microbicidal activity; phagocytosis; surgery.)**

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THE benefits of supplemental oxygen include extra time to resolve airway problems[1] and a twofold reduction in the incidence of postoperative nausea and vomiting.[2] However, high concentrations of inspired oxygen are also associated with atelectasis. Atelectasis results in part from uptake of oxygen from isolated alveoli, an effect that is more pronounced at high-oxygen partial pressures. Administration of 100% oxygen produces atelectasis in the immediate postoperative period,[3,4] although the extent to which this atelectasis impairs pulmonary function and gas exchange is controversial.[3–6] Our first goal was therefore to test the hypothesis that 100% inspired oxygen maintained for approximately 8 h intraoperatively is not associated with impaired pulmonary oxygenation.

Oxidative killing is the primary mechanism of inactivating bacteria. Oxidative killing depends on the produc-

tion of bactericidal superoxide radical from molecular oxygen, and the rate of this reaction, catalyzed by the NADPH-linked (or "primary") oxygenase, is therefore dependent on partial pressure of oxygen (P_{O_2}). As might be expected, resistance to surgical wound infection depends highly on skin and muscle oxygen availability throughout the clinically observed range of tissue oxygen tensions.[7] The importance of this mechanism was recently confirmed by a study that showed that supplemental perioperative oxygen administration decreases the incidence of surgical wound infection by half.[8]

Alveolar immune cells, of which more than 90% are macrophages, are the first line of pulmonary defense. The role of oxygen in resistance to infection during and after anesthesia and surgery is perhaps more complicated in the lungs. One reason is that oxygen partial pressure in the lungs far exceeds precapillary arterioles. For example, oxygen *per se,* inhalation of volatile anesthetics, and mechanical ventilation also provoke inflammatory reactions that are manifested by expression of genes for proinflammatory cytokines.[9-15] Antimicrobial functions, such as phagocytic and bactericidal activities of alveolar macrophages, are seriously impaired by anesthesia and surgery.[14-21] We observed a significant time-dependent decrease in antimicrobial functions during anesthesia and surgery.[17-19] Smoking, which reduces pulmonary inflammatory responses, further impairs intraoperative antimicrobial function.[18,19] To the extent that supplemental oxygen provokes an inflammatory response, activation of proinflammatory cytokines may help to preserve normal phagocytic and bactericidal activities in alveolar macrophages.[22-24]

Our second goal was to test the hypothesis that 100% inspired oxygen activates pulmonary inflammatory systems and prevents the reduction in phagocytic and microbicidal activities. To this end, we evaluated a number of cellular functions including (1) "opsonized" and "unopsonized" phagocytosis, (2) microbicidal activity, (3) macrophage aggregation, and (4) neutrophil influx. Also we evaluated gene expression of proinflammatory cytokines, including interleukin (IL)-1β, IL-6, IL-8, interferon-γ (IFN-γ), and tumor necrosis factor α (TNF-α) in alveolar immune cells.

Methods

The protocol of this study was approved by the Institutional Review Board at the University of Hirosaki. We explained possible harmful effects of bronchoalveolar lavage and intraoperative inhalation of 100% oxygen, and written informed consent was obtained from all participating patients. We observed 60 patients who were scheduled to undergo orthopedic surgery exceeding 6 h in duration, as in our previous study.[17]

We excluded patients based on one or more of the following conditions: (1) presence of chronic obstructive or restrictive pulmonary disease; grading of American Society of Anesthesiologists (ASA) physical status II or higher; (2) current use of steroid or nonsteroidal antiinflammatory medications; (3) presence of pulmonary or other infection or abnormal chest radiography results; (4) presence of neoplastic disease; (5) a forced vital capacity and forced expiratory volume in 1 s less than 80% and 70% of the predicted value, respectively; (6) a body mass index exceeding 30; or (7) a history of smoking within 8 weeks preoperatively.

Protocol

Anesthesia was induced with propofol (1.5–2 mg/kg), fentanyl (1–3 μg/kg) and vecuronium (0.08–0.1 mg/kg). Anesthesia was also maintained with propofol (5–8 mg $\cdot$ $kg^{-1} \cdot h^{-1}$), fentanyl (10–20 μg/kg) and vecuronium (2–3 mg/h). A volume-controlled ventilator set to 10 ml/kg was used for mechanical ventilation. The respiratory rate (10–15 breaths/min) was controlled to produce arterial partial pressure of carbon dioxide (P_{CO_2}) between 35 and 45 mmHg. Inspiratory–expiratory ratio was 0.5 and positive end-expiratory pressure was not given. Radial arterial pressure, the electrocardiography, and pulse oximeter saturation were monitored in all patients. A catheter was inserted *via* the right internal jugular vein to monitor central venous pressure.

After induction of anesthesia, patients were assigned to two treatment groups. The computer-generated assignments were kept in sealed, sequentially numbered envelopes until use.

For auditing purposes, both the assignment and the envelope number were recorded. The groups were as follows: (1) 30% oxygen and 70% nitrogen and (2) 100% oxygen.

All patients were transported to the recovery unit immediately after surgery. The patients were administered 40% oxygen *via* a non-rebreathing mask throughout recovery and until 8:00 AM the subsequent morning. Subsequently, all patients breathed room air. Supplemental oxygen was administered to patients in either group as necessary to maintain a pulse oximeter saturation of at least 95%.

Evaluation of Pulmonary Function and Complications

Arterial blood was sampled for each patient for gas analysis before each bronchoalveolar lavage, 1 h after extubation while breathing 40% oxygen, and approximately 24 h after extubation while breathing room air. The saturation from a pulse oximeter, end-tidal P_{CO_2}, and peak airway pressure were measured at 30-min intervals during anesthesia. Saturation and blood pressure were recorded every 30 min during recovery and at 1-h intervals on the ward.

As a control, preoperative anterior-posterior and lateral chest radiographs were obtained while the patient was in the supine position. Additional anterior-posterior and lateral chest radiographs were obtained while the patient was in the supine position on the first postoperative day. All postoperative measurements were performed before patients were mobilized and before they first underwent chest physical therapy. The anesthesiologist and investigator assigned to perioperative treatment were aware of the group assignment. However, patients, nurses, surgeons, and other investigators were blind to the group assignments.

Postoperative pulmonary complications were evaluated by a physician who was unaware of the patient's group assignment and intraoperative treatment. This investigator was also blind to the study results. Postoperative pulmonary complications were divided into major and minor categories, as suggested by Bluman *et al.*[25] Major complications included pulmonary infection documented by chest radiograph and associated with core temperatures exceeding 38.5°C, reintubation associated with respiratory failure, and hospital readmission because of pneumonia. Minor complications consisted of slight respiratory difficulty (mild dyspnea or tachypnea without abnormal results of blood gas analysis), coughing, unexpected postoperative use of aerosol treatment, or new or worsening atelectasis seen on the postoperative chest radiograph.

Bronchoalveolar Lavage and Blood Sampling

As previously described,[15,17,19] bronchoalveolar lavages were performed immediately after induction, 2, 4, and 6 h after induction of anesthesia, and at the end of the surgery. A bronchovideoscope (BF type P200; CV200; CLV-U20D; Olympus Co., Tokyo, Japan) was placed through the endotracheal tube while maintaining mechanical ventilation. The tip of the bronchovideoscope was wedged into a left or right segment of the lower or middle lobe. This segment was then lavaged *via* the suction port after instilling 20 ml sterile saline solution that contained NaCl (125 mM), KCl (6 mM), dextrose (10 mM), HEPES (20 mM), and lidocaine (16 mM) titrated with NaOH to a pH of 7.4. The lavage fluid was then gently aspirated. This procedure was repeated 5 times until the total instillation of solution was 100 ml. A different randomly chosen segment was lavaged at each time point; the same investigator performed all the bronchoalveolar lavages.

After straining through a single layer of loose cotton gauze to remove mucus, we pooled the lavage fluid and counted the number of alveolar macrophages and determined the viability of alveolar cells. Cell differentiation and aggregation were evaluated by counting 500 cells on a Wright-Giemsa stained slide. We previously described this method in detail.[14-19] Lavage fluid was then divided into three equal volumes for determination of phagocytosis, bactericidal activity, and gene expression of proinflammatory cytokines by use of reverse-transcription polymerase chain reaction (PCR).

Expression of proInflammatory Cytokine Genes

The following molecular analysis of proinflammatory cytokines was based on our previously reported method.[15,19] After separation of alveolar cells by centrifugation, the cell pellets were dissolved immediately in 0.5 ml guanidinium buffer solution (4 M guanidinium isothiocyanate, 50 mM Tris-HCl, 10 mM EDTA, 2% sarcoryl, 100 mM mercaptoethanol). RNA was isolated using the well-established acid guanidinium-phenol-chloroform method. We obtained 2.7-5.5 μg RNA from each sample. cDNA was synthesized at 40°C for 60 min from 2.5 μg RNA using a 20-μl total reaction mixture, which included Tris-HCl buffer (pH 8.3), 1 mM deoxyribonucleic triphosphates, and 0.125 μM oligo dT primers, and 20 U ribonuclease inhibitor and 0.25 U reverse transcriptase. The reverse transcriptase was inactivated by heating to 95°C for 2 min at the end of synthesis.

The semiquantitative reverse-transcription PCR mixture (50 μl) contained cDNA synthesized from 0.5 μg RNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM deoxyribonucleic triphosphate, 0.2 μM 5′ and 3′ oligonucleotide primers, and 2.5 U DNA polymerase (Takara, Co., Tokyo, Japan). The reaction mixture was then 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 (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 experi-

ments so that the amplification process was performed during the exponential phase of amplification. The number of PCR cycles are as follows: 26 for β-actin, 29 for IL-1β, 35 for IL-6, 32 for IFN-γ, and 27 for IL-8 and TNF-α. We used the same sequence of cytokine-specific primer pairs as in our previous study.[15] Coamplification of the cDNA for each cytokine and β-actin was then 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 the β-actin. The PCR products were separated using electrophoresis on a 1.8% agarose gel containing 0.5 μg/ml ethidium bromide. PCR products were visualized on a transilluminator (model FBTIV-816; Fisher Scientific, Pittsburgh, PA) using a 312-nm wavelength and photographed with use of Polaroid 667 film (Japan Polaroid, Tokyo, Japan). The band images were obtained by scanning the photograph using a ScanJet 3P (Hewlett-Packard, Cupertino, CA). The total intensity (average intensity × total pixels) of each band was measured with use of Mocha software (Jandel Scientific Software, San Rafael, CA). To evaluate the relative amount of cytokine mRNA in each sample, the cytokine: β-actin ratio of the intensity of ethidium bromide luminescence for each PCR product was calculated.

Phagocytic and Microbicidal Activities

Phagocytic and microbicidal activities were evaluated as previously described.[17,19] We started these analyses within 15 min, after the cell harvest. Alveolar macrophages were separated from bronchoalveolar lavage fluid by centrifugation at 200*g* for 10 min. After the supernatant was decanted, alveolar macrophages were resuspended at a concentration of 0.5×10^6 cells/ml in a balanced saline solution containing NaCl (125 mM), KCl (6 mM), dextrose (10 mM), $CaCl_2$ (0.3 mM), and $MgCl_2$ (1.0 mM), titrated with NaOH to a pH of 7.4.

Resuspended alveolar macrophages were incubated as suspensions at 37°C in 20-ml sterile centrifuge tubes on a shaking platform (60 cycles/min). Unopsonized and opsonized (1.0-μm diameter) particles were added to the separate centrifuge tubes, each containing a sample of the cell suspension; the particle-to-cell ratios were 15:1. The cell suspension was placed on a glass slide, fixed, and stained. We recorded the fraction that ingested at least one particle and the number of fluorescent particles per positive phagocytic alveolar macrophage.

Bactericidal ability of the alveolar macrophages was determined by their ability to kill *Listeria* monocytogenes using a previously described method.[17,19] 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 (Gibco BRL, Life Tech. Inc., Rockville, MD). After nonadherent cells were removed by washing with RPMI-1640, the remaining cells (> 98% macrophages) were resuspended in 0.5 ml RPMI containing 10% normal human serum.

The bacteria were resuspended in the same medium at a concentration of 2×10^6 colony-forming units (cfu)/ml. Resuspended aliquots of *Listeria* (0.5 ml) were mixed with the alveolar macrophages and incubated for 30 and 120 min in 5% CO_2-air. Centrifuged pellets of alveolar macrophages were lysed by adding 10 ml sterilized distilled water and vortexing for 30 s to release bacteria. The viable fraction of *Listeria* bacteria was determined by plating serial 10-fold dilutions 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.

Data Analysis

Immediately after induction of anesthesia was designated as elapsed time zero. Time-dependent intragroup data were evaluated using two-way analysis of variance and *post-hoc* Dunnett tests for comparison with elapsed time zero; $P < 0.05$ was considered to be statistically significant. Differences between groups at each time point were evaluated using two-tailed, unpaired *t* tests. Our nominal *P* value was 0.05. Because we compared values in the two groups at five time points, $P < 0.01$ was considered to be statistically significant. Data are expressed as the mean ± SD.

Results

Demographic and morphometric characteristics were similar in the two groups (table 1). Various hemodynamic and physiologic responses differed progressively time in each group. However, there were no significant differences between the patients administered 30% and those administered 100% oxygen (table 2).

Preoperative pulmonary functions were comparable in the two groups. Intraoperative arterial oxygen partial pressure differed significantly in the two groups, as might be expected from their differing inspired oxygen

Table 1. Morphometric and Demographic Characteristics, Pulmonary Status, and Anesthetic Management

	30%	100%
Number	30	30
Age (yr)	48 ± 9	49 ± 8
Gender (M/F)	16/14	19/11
Weight (kg)	59 ± 9	60 ± 7
Height (cm)	163 ± 7	165 ± 8
ASA physical status (I/II)	9/21	12/18
Mean arterial pressure (mmHg)	93 ± 12	96 ± 13
Heart rate (beats/min)	78 ± 13	76 ± 12
Cardiac index ($l \cdot min^{-1} \cdot m^{-2}$)	3.0 ± 0.3	3.1 ± 0.3
FVC (% predicted)	96 ± 10	97 ± 14
FEV_1 (% predicted)	87 ± 8	88 ± 6
Duration of anesthesia (h)	8.4 ± 1.1	8.1 ± 0.8
Total fentanyl (mg)	0.8 ± 0.2	0.8 ± 0.2

Averages are presented as the mean ± SD; there were no statistically significant differences between the groups.

ASA = American Society of Anesthesiologists; FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1 s.

concentrations. Arterial gas partial pressures and partial pressure of arterial oxygen (Pa_{O_2})-fraction of inspired oxygen (FI_{O_2}) were also comparable in the two groups while the patient was in the recovery room and on the first postoperative day. From 8:00 AM the subsequent day, no supplemental oxygen was needed. Atelectasis was not identified on any preoperative chest radiographs. However, mild atelectasis was observed in 17% (5 of 30) of the patients in the 30% oxygen group and in 23% (7 of 30) of the patients administered 100% oxygen (P = NS). Clinical follow-up failed to identify pneumonia or other respiratory complications in the study patients (table 3).

There were no statistically significant differences in the recovery rates and concentration of alveolar cells as a function of time within or between groups. Also, there were no differences in viability of alveolar cells between two groups. However, the percentage of neutrophils increased significantly over time, whereas the fraction of macrophages decreased significantly. The percentage of lymphocyte did not change. The fraction of neutrophils increased twice as much in patients administered 100% than in those administered 30% oxygen, starting 4 h after anesthesia. The fraction of aggregated cells increased over the study period in both groups. However, the increase in aggregation was slightly, but significantly, greater in patients administered 100% oxygen than in those administered 30% only at the end of surgery (table 4).

The relative amount of mRNA for all cytokines was minimal at elapsed time zero. In both groups, expression of all cytokines except IL-6 increased 2 h after anesthesia. The increases in gene expression of IL-8 and TNF-α were greater in patients administered 100% oxygen than in those administered 30%, starting 2 h after anesthesia. At the end of surgery, the increases in gene expression of IL-8 and TNF-α were 3 and 20 times greater in patients administered 100% oxygen than in those administered 30%. Also, the increases in gene expression of IFN-γ and IL-1β were twice as great in patients administered 100% oxygen than in those administered 30%, starting 4 and 6 h after anesthesia, respectively. A slight, but statistically significant, increase in gene expression for IL-6 was detected in patients who were administered 100% oxygen for 6 h, whereas no increase was detectable in patients administered 30% oxygen (fig. 1)

Starting 4 h after anesthesia, unopsonized phagocytosis decreased significantly in both groups. Unopsonized phagocytosis decreased 30% in patients administered 30% oxygen by the end of surgery, but only 15% in patients administered 100% oxygen. There was also significantly less reduction in opsonized phagocytosis in patients administered 100% oxygen than in those administered 30% (fig. 2).

Microbicidal activity, as evaluated by killing of *Listeria* monocytogenes at 30 and 120 min postincubation, decreased significantly starting 4 and 6 h after anesthesia in patients administered 30 and 100% oxygen, respectively. By the end of surgery, microbicidal activity decreased by approximately 20% in patients who were administered 30% oxygen, whereas it only decreased by approximately 10% in those administered 100% oxygen (fig. 3).

Discussion

Atelectasis

We used Pa_{O_2}-FI_{O_2} for evaluating pulmonary oxygenation. The Pa_{O_2}-FI_{O_2} is augmented at high inspired oxygen concentrations. It is therefore not surprising that the Pa_{O_2}-FI_{O_2} in our patients was increased during inhalation of 40% oxygen in the postanesthesia care unit. However, there were no significant differences between patients administered 30% and those administered 100% intraoperative oxygen. Furthermore, both the Pa_{O_2}-FI_{O_2} and the incidence of atelectasis, as determined by radiography, were comparable in each group on the first postoperative day.

Our recent study showed that pulmonary function tests and computerized tomography were used to show

Table 2. Intraoperative Data

		0	2	4	6	End	Recovery
MAP (mmHg)	30%	75 ± 10	91 ± 13*	87 ± 13*	87 ± 12*	88 ± 13*	91 ± 13*
	100%	77 ± 9	93 ± 13*	94 ± 13*	92 ± 13*	91 ± 14*	94 ± 12*
HR (beats/min)	30%	70 ± 13	79 ± 16*	79 ± 14*	77 ± 14*	79 ± 13*	77 ± 13*
	100%	69 ± 11	77 ± 11*	83 ± 13*	81 ± 14*	81 ± 11*	75 ± 14*
T_{core} (°C)	30%	36.7 ± 0.4	36.3 ± 0.4	36.4 ± 0.4	36.4 ± 0.4	36.3 ± 0.5	—
	100%	36.6 ± 0.3	36.3 ± 0.4	36.3 ± 0.5	36.2 ± 0.5	36.2 ± 0.6	—
PIP (cm H_2O)	30%	15 ± 3	16 ± 2	15 ± 2	16 ± 2	16 ± 2	—
	100%	16 ± 3	15 ± 3	16 ± 2	16 ± 2	16 ± 2	—
pH	30%	7.42 ± 0.03	7.40 ± 0.04	7.39 ± 0.03	7.38 ± 0.03	7.38 ± 0.03	7.36 ± 0.03
	100%	7.43 ± 0.03	7.41 ± 0.04	7.40 ± 0.04	7.38 ± 0.04	7.36 ± 0.05	7.35 ± 0.05
Pa_{CO_2} (mmHg)	30%	39 ± 3	39 ± 2	40 ± 3	39 ± 2	39 ± 2	43 ± 5
	100%	40 ± 3	40 ± 2	40 ± 2	40 ± 2	40 ± 2	41 ± 4
Ca^{2+} (mM)	30%	1.09 ± 0.06	1.08 ± 0.06	1.09 ± 0.06	1.08 ± 0.05	1.07 ± 0.05	1.07 ± 0.06
	100%	1.09 ± 0.05	1.09 ± 0.05	1.10 ± 0.04	1.10 ± 0.04	1.09 ± 0.05	1.07 ± 0.06
Mg^{2+} (mM)	30%	0.51 ± 0.04	0.51 ± 0.04	0.49 ± 0.04*	0.48 ± 0.05*	0.46 ± 0.05*	0.46 ± 0.05*
	100%	0.52 ± 0.04	0.52 ± 0.04	0.51 ± 0.04*	0.49 ± 0.04*	0.48 ± 0.05*	0.47 ± 0.05*
BIS	30%	55 ± 6	58 ± 5	55 ± 6	57 ± 5	57 ± 5	91 ± 3*
	100%	53 ± 6	55 ± 6	57 ± 5	55 ± 4	55 ± 4	91 ± 3*

Results are presented as means ± SDs.

* Statistically significant differences ($P < 0.05$) from elapsed time 0.

MAP = mean arterial pressure; HR = heart rate; T_{core} = esophageal temperature; PIP = peak inspiratory pressure; Pa_{CO_2} = arterial carbon dioxide tension; BIS = the Bispectral Index of the electroencephalogram.

that 80% perioperative oxygen does not increase the incidence of atelectasis on the first postoperative morning.[26] Although atelectasis detected by computed tomography is not the same as that detected by chest radiography, there were no differences in oxygenation between groups. Evidence therefore suggests that administration of 80–100% oxygen is not associated with persisting atelectasis until the first postoperative day—even when the duration of surgery extends to 8 h.

Expression of proInflammatory Cytokines

Anesthesia and surgery augmented gene expression of IL-1β, IL-8, TNF-α, and IFN-γ in alveolar macrophages after 2–4 h of anesthesia, as in our previous studies.[14,15,19] The increases in gene expression for all cytokines were considerably greater in patients administered 100% oxygen than in those administered 30%.

Tumor necrosis factor α is a potent proinflammatory cytokine in the macrophage-monocyte-neutrophil sys-

Table 3. Pulmonary Function and Atelectasis

Groups	Times	30%	100%	*P*
Pa_{O_2} (mmHg)		86 ± 8	87 ± 10	NS
Pa_{CO_2} (mmHg)	Preanesthetic	41 ± 3	40 ± 4	NS
Pa_{O_2}/FI_{O_2}		411 ± 40	415 ± 46	NS
Pa_{O_2} (mmHg)	Intraoperative (0 h)	136 ± 25	441 ± 43	<0.0001
Pa_{O_2} (mmHg)	(2 h)	136 ± 23	440 ± 43	<0.0001
Pa_{O_2} (mmHg)	(4 h)	138 ± 22	438 ± 33	<0.0001
Pa_{O_2} (mmHg)	(6 h)	134 ± 22	449 ± 36	<0.0001
Pa_{O_2} (mmHg)	(End)	135 ± 19	431 ± 42	<0.0001
Pa_{O_2} (mmHg)	Recovery	135 ± 40	132 ± 37	NS
Pa_{O_2}/FI_{O_2}		644 ± 191	627 ± 174	NS
Pa_{O_2} (mmHg)		83 ± 10	81 ± 12	NS
Pa_{CO_2} (mmHg)	Postoperative	40 ± 3	39 ± 3	NS
Pa_{O_2}/FI_{O_2}		393 ± 49	389 ± 56	NS
Atelectasis (%)		17	23	NS

Pa_{O_2}/FI_{O_2} is a respiratory index. Preanesthetic values were obtained before induction of anesthesia. Recovery values were obtained 1 h after extubation while patients breathed 40% oxygen. Postoperative values were obtained approximately 24 h after surgery while patients breathed room air. Data are presented as means ± SDs.

NS = lack of statistical significance between the two groups.

Table 4. Cell Recovery and Distribution from Bronchoalveolar Lavage

		0 h	2 h	4 h	6 h	End
Recovery rate (%)	30%	65 ± 9	64 ± 9	64 ± 9	64 ± 7	63 ± 8
	100%	66 ± 9	64 ± 8	62 ± 7	62 ± 9	62 ± 10
Cells ($\times 10^4/cm^3$)	30%	16 ± 7	16 ± 3	16 ± 4	17 ± 5	17 ± 5
	100%	15 ± 6	14 ± 5	16 ± 6	16 ± 5	15 ± 6
Macrophage (%)	30%	91 ± 3	90 ± 4	89 ± 4*	86 ± 5*	83 ± 7*
	100%	91 ± 3	88 ± 3*†	85 ± 4*†	80 ± 4*†	75 ± 7*†
Lymphocyte (%)	30%	8 ± 3	8 ± 3	7 ± 4	7 ± 4	8 ± 4
	100%	8 ± 3	8 ± 3	8 ± 4	8 ± 4	8 ± 5
Neutrophils (%)	30%	1 ± 1	2 ± 3	4 ± 2*	7 ± 3*	9 ± 5*
	100%	1 ± 1	4 ± 2*†	7 ± 2*†	12 ± 3*†	16 ± 4*†
Viability (%)	30%	94 ± 3	95 ± 3	94 ± 3	94 ± 3	94 ± 2
	100%	94 ± 2	93 ± 2	94 ± 3	94 ± 2	93 ± 2
Aggregation (%)	30%	3.7 ± 3.0	4.4 ± 4.4	8.2 ± 4.1*	11.8 ± 5.0*	15.4 ± 6.4*
	100%	4.0 ± 3.1	6.5 ± 3.8	10.8 ± 5.8*	15.4 ± 7.4*	22.7 ± 8.9*†

Data expressed as means ± SDs.

* Statistically significant differences ($P < 0.05$) from elapsed time 0.

† Significant differences ($P < 0.01$) between patients administered 30% and 100% oxygen.

tem. Horinouchi *et al.*[11] reported that only 3 h of exposure to 100% oxygen induces gene expression of TNF-α in alveolar macrophages. Burges *et al.*[9] similarly reported that exposure to hyperoxia produced IL-1β, IL-6, and TNF-α in alveolar macrophages. In our patients, the increase in gene expression of TNF-α was 20 times greater in patients administered 100% oxygen than in those administered 30%, starting 2 h after anesthesia. Our results suggest that hyperoxia markedly increases expression of TNF-α and activates proinflammatory responses.

The pharmacologic action of IL-1β is very similar to that of TNF-α. However, the increase in IL-1β gene in patients administered 100% oxygen was at most twice as much as in those administered 30%, and there was no difference until 4 h of anesthesia. We previously reported that alveolar macrophages did not produce IL-1β genes 2 h after mechanical ventilation, but that expression of TNF-α genes was easily detectable.[14] Alveolar macrophages can secrete more TNF-α, but less IL-1β, than do their precursors, plasma monocytes.[27] These results suggest that IL-β is less sensitive to hyperoxic proinflammatory responses than is TNF-α.

Interleukin-8 plays an important role in hyperoxia-induced proinflammatory responses because increased expression of IL-8 genes continued for up to 48 h of hyperoxic exposure, whereas expression of other cytokines, including TNF-α, IL-1β, and IL-6, were simultaneously suppressed.[12] Deaton *et al.*[13] reported that IL-8 released by alveolar macrophages in response to hyperoxia by alveolar macrophages is extremely biologically

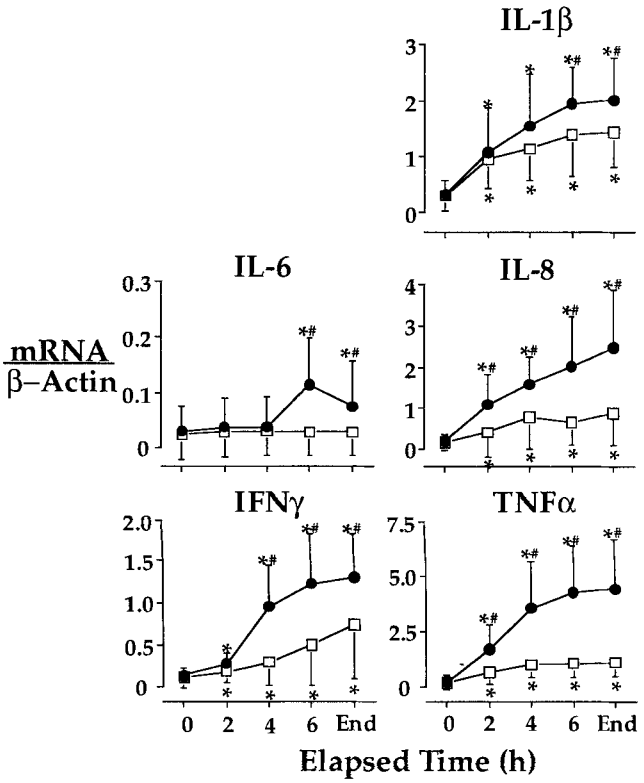

Fig. 1. Expression of proinflammatory cytokines during anesthesia with 100% (n = 30, circles) or 30% (n = 30, squares) inspired oxygen. Ratios of cytokine mRNA to β-actin mRNA are presented as the mean ± SD. *Statistically significant differences ($P < 0.05$) from elapsed time zero in each group; #statistically significant difference ($P < 0.01$) between the two groups.

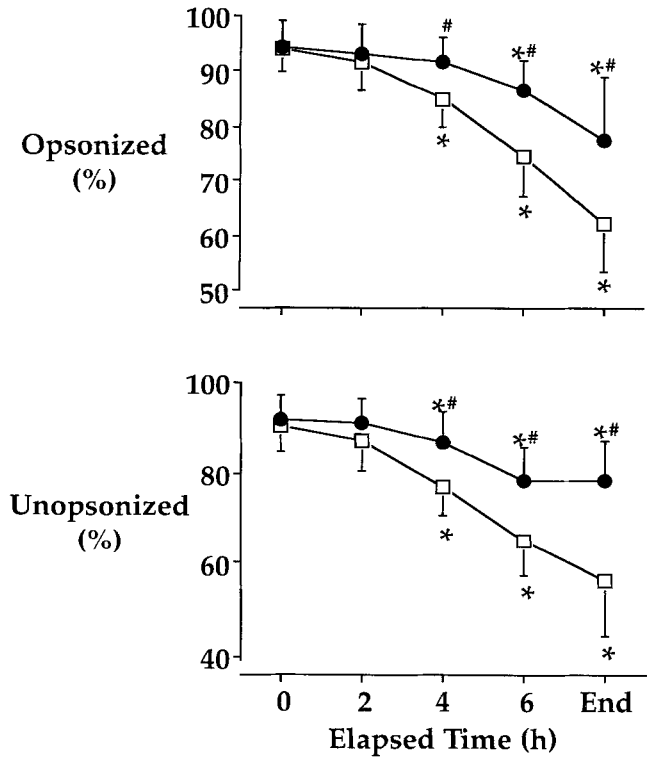

Fig. 2. The fraction of alveolar macrophages ingesting opsonized and nonopsonized particles during anesthesia with 100% (n = 30, circles) and 30% (n = 30, squares) inspired oxygen. *Statistically significant differences ($P < 0.05$) from elapsed time zero in each group; #significant differences ($P < 0.01$) between the two groups. Data are expressed as the mean ± SD.

active, as indicated by its ability to recruit neutrophils. These findings presumably explain why neutrophil influx was greater in patients administered 100% oxygen than in those administered 30% oxygen.

A notable finding is that increases in gene expression for IL-6, starting from 6 h after anesthesia, were significantly greater in patients administered 100% oxygen than in those administered 30%. We previously reported that there was little or no expression of the gene for IL-6 in alveolar leukocytes after general anesthesia in our human and animal studies.[14,15,18,19] Because operative procedure and anesthetic management were the same in our current and previous study, inhalation of 100% oxygen is the most plausible factor contributing to the increases of IL-6 expression in alveolar cells. In contrast, both gene expression and production of IL-6 increase after cardiopulmonary bypass.[20,21] Cardiopulmonary bypass is well-known to cause pulmonary inflammation. Our data also suggest that inhalation of 100% oxygen augments proinflammatory responses in these patients.

The duration of hyperoxic exposure is clearly an important determinant of cytokine responses. Numerous harmful pulmonary effects have been observed after prolonged hyperoxic exposure (*i.e.*, > 24 h). Increases in the number of alveolar leukocytes in the distal airway, an initial histologic feature of hyperoxic pulmonary injury,[28] are observed 72 h after hyperoxic exposure. Desmarquest *et al.*[12] reported that gene expression of proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, decreased 48 h after hyperoxic exposure, but that gene expression of these cytokines increased during the first hour of exposure. They concluded that the decrease, rather than the increase, in gene expression of TNF-α, IL-1β, and IL-6 contributes to the development of hyperoxic pulmonary injury. Our patients were exposed to 100% oxygen for up to 10 h. Augmentation in expression of the genes for proinflammatory cytokines may not be harmful during anesthesia.

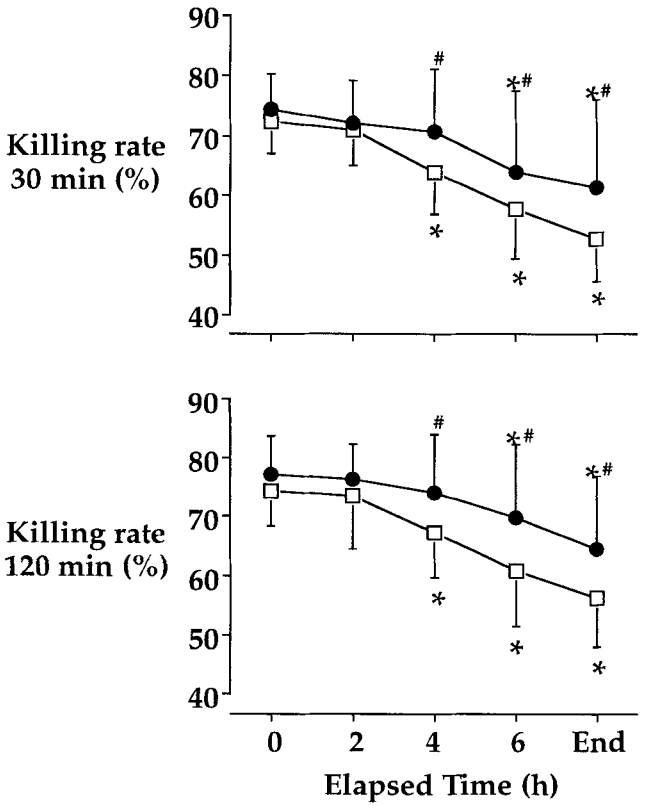

Fig. 3. The percentage of *Listeria* monocytogenes killed by alveolar macrophage during anesthesia with 100% (n = 30, circles) and 30% (n = 30, squares) inspired oxygen after 30 and 120 min incubation. *Statistically significant differences ($P < 0.05$) from elapsed time zero in each group; #significant differences ($P < 0.01$) between the two groups. Data are expressed as the mean ± SD.

Phagocytic and Microbicidal Activities

Phagocytic and microbicidal activities decreased progressively time in both groups, as reported previously.[17-19] These may be explained in part by general anesthesia and surgical stress. The increases in oxygen consumption by phagocytic and bactericidal activities are seriously inhibited by volatile and intravenous anesthetics.[29] In addition to anesthetic exposure itself, activation of the hypothalamic-pituitary-adrenal system as a result of surgical stress modulates immune responses. According to Dahanukar *et al.*[30] decreases in phagocytosis correlate with the increase in serum cortisol concentration during surgery. Phagocytosis is suppressed by epinephrine in a dose-dependent fashion.[31] This mechanism may be speculated about to explain the observed simultaneous decrease in phagocytic and microbicidal activities and the activation of proinflammatory responses.

Phagocytic and bactericidal activities of alveolar macrophages are key elements of pulmonary defense. One of our major findings is that phagocytic and microbicidal activities decreased only half as much in patients administered 100% oxygen as in those administered 30% oxygen. Although the *Listeria* monocytogenes were different from the majority of postoperative pulmonary infections, and the assay was performed in room air, supplemental oxygen may augment antimicrobial activity during anesthesia and surgery. Augmented expression of proinflammatory cytokines by supplemental oxygen is important because phagocytic and microbicidal activities are up-regulated by many proinflammatory cytokines and growth factors.[22-24]

Interestingly, some studies report that hyperoxia decreases phagocytic and bactericidal activities, which contrasts with our findings.[32,33] One explanation for these apparently contradictions may be that the exposure time in these studies exceeded 24 h. This exposure duration is known to cause direct toxicity to alveolar macrophages and prompts them to defend themselves by producing superoxide dismutase. Superoxide dismutase, in turn, decreases oxidative bactericidal activity.[34]

Neutrophil Influx, Macrophage Aggregation, and Viability

The increases in neutrophil influx and macrophage aggregation indicate proinflammatory responses. Neutrophil influx and macrophage aggregation was significantly augmented in patients administered 100% oxygen, a result that was expected based on previous work.[35,36] Various immunologic mediators could have facilitated these responses. For example, IL-8 is among the most potent chemoattractant for neutrophils, and we documented increased expression of the IL-8 gene. It is likely that adhesion molecules of the macrophage-monocyte-neutrophil system[9,37] contribute to neutrophil influx and macrophage aggregation. Hyperoxic exposure considerably causes macrophage aggregation.[38]

Viability did not change during the entire course of anesthesia. According to Bravo Cuellar *et al.*,[39] it takes 3 days for hyperoxic exposure to decrease viability of alveolar macrophages. These results suggest that inhalation of 100% oxygen is not toxic to alveolar macrophages, even when exposure exceeds 8 h.

Conclusion

Antimicrobial and proinflammatory responses of alveolar macrophages, at both cellular and histologic levels, were markedly augmented in patients administered 100% oxygen, compared with those administered 30% oxygen, during anesthesia and surgery. We chose healthy patients without risk factors for postoperative pulmonary complications. Increased antimicrobial function may be beneficial for pulmonary defense. The increases in gene expression of proinflammatory cytokines indicate activation of pulmonary defenses during anesthesia.[40] In contrast, excessive activation of immune response can produce pulmonary injury, and hyperoxic exposure exacerbates serious pulmonary inflammation.[9,10,41,42] Whether we observed an appropriate defense against pulmonary insult or a harmful inflammatory reaction remains to be determined. From our study, we did not find the clinical relevance of observed findings. We conclude that intraoperative inhalation of 100% oxygen augments antimicrobial and proinflammatory responses.

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