

Smoking Decreases Alveolar Macrophage Function during Anesthesia and Surgery

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Background: Smoking changes numerous alveolar macrophage functions and is one of the most important risk factors for postoperative pulmonary complications. The current study tested the hypothesis that smoking impairs antimicrobial and proinflammatory responses in alveolar macrophages during anesthesia and surgery.

Method: The authors studied 30 smoking and 30 nonsmoking patients during propofol–fentanyl general anesthesia. Alveolar immune cells were harvested by bronchoalveolar lavage immediately and 2, 4, and 6 h after induction of anesthesia and at the end of surgery. The types of alveolar immune cell and macrophage aggregation were determined. The authors measured opsonized and unopsonized phagocytosis. Microbicidal activity was determined as the ability of the macrophages to kill *Listeria monocytogenes* directly. Finally, RNA was extracted from harvested cells and cDNA was synthesized by reverse transcrip-

tion. The expression of interleukin 1 β , 6, and 8, interferon γ , and tumor necrosis factor α were measured by semiquantitative polymerase chain reaction using β -actin as an internal standard.

Results: The fraction of aggregated macrophages increased significantly over time in both groups, whereas phagocytosis of opsonized and nonopsonized particles and microbicidal activity of alveolar macrophages decreased significantly. The changes, though, were nearly twice as great as in patients who smoked. Gene expression of all proinflammatory cytokines in alveolar immune cells except interleukin 6 increased 2- to 20-fold over time in both groups. The expression of interleukin 1 β , interferon γ , and tumor necrosis factor α , however, increased only half as much in smokers as in nonsmokers.

Conclusion: Smoking was associated with macrophage aggregation but markedly reduced phagocytic and microbicidal activity—possibly because expression of proinflammatory cytokines was reduced in these patients. Our data thus suggest that smokers may have a limited ability to mount an effective pulmonary immune defense after anesthesia and surgery. (Key words: aggregation; gene expression; microbicidal activity; phagocytosis; proinflammatory cytokines; pulmonary.)

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ALVEOLAR immune cells, 90% of which are macrophages, are the first line of pulmonary defense. Among their major antimicrobial functions are chemotaxis, phagocytosis, and microbicidal activity against foreign invaders. Alveolar macrophages also have proinflammatory functions including immune modulation *via* production of proinflammatory cytokines and chemoattractants for neutrophils.

We previously reported a significant time-dependent decrease in antimicrobial functions including phagocytosis and microbicidal activity of alveolar macrophages during anesthesia and surgery.^{1,2} In contrast, we found that macrophage aggregation and neutrophil influx are augmented during anesthesia and surgery.^{1–3} Inhalation of volatile anesthetics and mechanical ventilation each provokes inflammatory reactions.^{3,4} These data suggest that anesthesia and surgery provoke proinflammatory responses from alveolar macrophages.

Cigarette smoking also modulates both antimicrobial and proinflammatory functions in alveolar macrophages.

Smoking is one of the most important risk factors for postoperative pulmonary complications. For example, postoperative pulmonary complications are up to five times more common in smokers—even in the absence of underlying pulmonary disease or abnormal pulmonary function tests.^{5,6} Changes in alveolar macrophage function in smoking patients during anesthesia and surgery, however, have yet to be reported. We previously reported that both antimicrobial and proinflammatory functions decrease substantially in smoke-exposed rats during halothane and isoflurane anesthesia with mechanical ventilation.⁷ We therefore tested the hypothesis that chronic smoking also impairs both antimicrobial and proinflammatory functions in human alveolar macrophages during anesthesia and surgery.

Because alveolar macrophage function is complex, we evaluated a number of functions including opsonized and unopsonized phagocytosis, microbicidal activity, macrophage aggregation, and neutrophil influx. We also evaluated gene expression of proinflammatory cytokines including interleukin (IL) 1 β , IL-6, IL-8, interferon (IFN) γ , and tumor necrosis factor (TNF) α in whole alveolar immune cells.

Methods

The protocol of this study was approved by the Institutional Review Board at the University of Hiroaki, and written informed consent was obtained from all participating patients. We studied 60 patients with a scheduled duration of surgery exceeding 6 h. All patients were undergoing orthopedic surgery as in previous study.² We defined smokers as patients who currently were smoking more than 1 pack/day and had done so for at least 1 yr before surgery. Nonsmokers were defined as patients who had never smoked cigarettes for 1 yr or more. Smokers were allowed to smoke in a special hospital room until just before surgery.

We also excluded patients with one or more of the following conditions: (1) chronic obstructive or restrictive pulmonary disease, with classification of American Society of Anesthesiologists physical status II or higher; (2) current steroid or nonsteroidal antiinflammatory medications, with pulmonary or other infection or abnormal chest radiograph; (3) neoplastic disease, with forced vital capacity and forced expiratory volume in 1 s less than 80 and 70% of the predicted values, respectively; or (4) a body mass index exceeding 30.

Protocol

We evaluated 30 nonsmoking and 30 smoking patients. Anesthesia was induced and maintained with propofol (5–8 mg \cdot kg⁻¹ \cdot h⁻¹), fentanyl (10–20 μ g/kg), and vecuronium. During anesthesia, all the patients were mechanically ventilated with 30% oxygen and 70% nitrogen to maintain arterial carbon dioxide tension (Pa_{CO₂}) between 35 and 45 mmHg. Radial arterial pressure, electrocardiogram, and pulse oximeter saturation were monitored in all patients. A catheter was inserted *via* the right internal jugular vein to monitor central venous pressure.

Before each performance of bronchoalveolar lavage, arterial blood was sampled for gas analysis and calcium and magnesium concentrations. As previously described,^{2,3} bronchoalveolar lavage was performed immediately after induction of anesthesia, 2, 4, and 6 h after induction, and at the end of the surgery. A bronchovideoscope (BF type P200, CV200, CLV-U20D; Olympus, Tokyo, Japan) was introduced through the endotracheal tube while mechanical ventilation was maintained. 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 of sterile saline solution containing 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 then was aspirated gently. This procedure was repeated five times, so 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.

Pulmonary Complications

Postoperative pulmonary complications were evaluated by a physician investigator who was unaware of the patient's smoking history. This investigator also was blind to the study results. Postoperative pulmonary complications were divided into major and minor categories, as suggested by Bluman *et al.*⁶ Major complications included pulmonary infection documented on chest radiograph associated with core temperatures exceeding 38.5°C, reintubation associated with respiratory failure, and hospital readmission for pneumonia. Minor complications consisted of unexpected postoperative use of aerosol treatment or new or worsening atelectasis on postoperative chest radiograph.

Treatment of Lavage Fluid

After straining through a single layer of loose cotton gauze to remove mucus, we pooled the lavage fluid in a sterile siliconized container and counted the number of alveolar macrophages with a hemocytometer. The viability of alveolar cells was evaluated by their ability to exclude the 0.2% trypan blue. We have described this method in detail.¹⁻⁴

Cell differentiation and aggregation were examined by counting 500 cells on a Wright-Giemsa stained slide. Aggregation of the alveolar immune cells was expressed as percentage of nuclei in aggregated cells per 500 nuclei counted on two or three slide preparations. Lavage fluid then was divided into three equal volumes for determination of phagocytosis, bactericidal activity, and gene expression of proinflammatory cytokines by reverse transcription polymerase chain reaction (PCR).

Gene Expression of Proinflammatory Cytokines by Semiquantitative Reverse Transcription PCR

Alveolar macrophages were separated from bronchoalveolar lavage fluid by centrifugation at 200g for 10 min. After the supernatant was decanted, 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% sarcosyl, 100 mM mercaptoethanol). Molecular analysis of proinflammatory cytokines was based on our previously reported method.^{3,4} RNA was isolated from the guanidinium buffer by the well-established acid guanidinium-phenol-chloroform method.⁸

The amount of isolated RNA was measured by a spectrophotometer (Model DU-65, Beckman, Tokyo, Japan). We obtained 2.7–4.5 μ g RNA from each sample. By incubation at 40°C for 60 min, cDNA was synthesized from 2.5 μ g RNA with 20 μ l total reaction mixture including Tris-HCl buffer (pH 8.3), 1 mM deoxyribonucleoside triphosphate, and 0.125 μ M oligo dT primers, as well as 20 U RNase inhibitor and 0.25 U avian myeloblastosis virus reverse transcriptase. After 60 min incubation, the reverse transcriptase was inactivated by heating to 95°C for 2 min.

The semiquantitative reverse transcription PCR mixture (50 μ l) contained cDNA synthesized from 0.5 μ g RNA 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 0.2 mM dNTP, 0.2 μ M 5' and 3' oligonucleotide primers, and 2.5 U Taq polymerase (Takara, Tokyo, Japan). The reaction mixture then was amplified in a DNA thermocycler (Perkin-Elmer, 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 experiments so that the amplification process was carried out during the exponential phase of amplification. The number of PCR cycles is as follows: 26 for β -actin, 29 for IL-1 β , 35 for IL-6, 32 for IFN- γ , 27 for IL-8 and TNF- α . We used the same sequence of cytokine-specific primer pairs as in our previous study.³ Coamplification of the cDNA for each cytokine and β -actin then 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 separated by electrophoresis on a 1.8% agarose gel containing 0.5 μ g/ml ethidium bromide. PCR products were visualized on a transilluminator (model FBTIV-816, Fisher Scientific, Pittsburgh, PA) at a 312-nm wavelength and photographed with Polaroid 667 film (Japan Polaroid, Tokyo, Japan). The band images were obtained by scanning the photograph with a ScanJet 3P (Hewlett-Packard, Cupertino, CA). The total intensity (average intensity \times total pixels) of each band was measured with 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.³

Phagocytosis and Microbicidal Activity

Phagocytosis and microbicidal activity were evaluated as previously described.^{2,4} Alveolar macrophages were separated from bronchoalveolar lavage fluid by centrifugation at 200g 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), CaCl₂ (0.3 mM), and MgCl₂ (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 μ m 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 tubes were incubated for 15 min, and the phagocytosis then stopped by addition of 2 ml of ice-cold balanced saline solution. The cell suspension was placed on a glass slide, fixed, and stained. We recorded the fraction

Table 2. Potential Confounding Factors

| | Time (h) | 0 | 2 | 4 | 6 | End |
|--------------------------|------------|-------------|--------------|--------------|--------------|--------------|
| MAP (mmHg) | Smoking | 77 ± 9 | 93 ± 13* | 94 ± 14* | 92 ± 13* | 91 ± 14* |
| | Nonsmoking | 75 ± 10 | 91 ± 14* | 87 ± 13* | 87 ± 12* | 88 ± 13* |
| HR (beats/min) | Smoking | 69 ± 11 | 78 ± 13* | 83 ± 13* | 81 ± 14* | 81 ± 11* |
| | Nonsmoking | 70 ± 13 | 79 ± 16* | 79 ± 15* | 77 ± 14* | 79 ± 12* |
| pH | Smoking | 7.41 ± 0.03 | 7.39 ± 0.03* | 7.37 ± 0.03* | 7.34 ± 0.04* | 7.31 ± 0.03* |
| | Nonsmoking | 7.42 ± 0.03 | 7.40 ± 0.03* | 7.37 ± 0.04* | 7.33 ± 0.03* | 7.31 ± 0.04* |
| PaO ₂ (mmHg) | Smoking | 136 ± 27 | 136 ± 23 | 138 ± 22 | 134 ± 22 | 135 ± 19 |
| | Nonsmoking | 130 ± 22 | 130 ± 25 | 130 ± 22 | 130 ± 22 | 131 ± 22 |
| PaCO ₂ (mmHg) | Smoking | 39 ± 3 | 39 ± 3 | 39 ± 2 | 39 ± 2 | 39 ± 2 |
| | Nonsmoking | 40 ± 3 | 40 ± 2 | 40 ± 2 | 40 ± 2 | 40 ± 2 |
| T _{core} (°C) | Smoking | 36.6 ± 0.3 | 36.3 ± 0.4* | 36.2 ± 0.5* | 36.3 ± 0.5* | 35.9 ± 0.6* |
| | Nonsmoking | 36.7 ± 0.3 | 36.3 ± 0.4* | 36.4 ± 0.5* | 36.3 ± 0.5* | 36.0 ± 0.6* |
| Ca ²⁺ (mM) | Smoking | 1.09 ± 0.06 | 1.08 ± 0.06 | 1.09 ± 0.06 | 1.08 ± 0.05 | 1.07 ± 0.05 |
| | Nonsmoking | 1.09 ± 0.05 | 1.09 ± 0.05 | 1.10 ± 0.04 | 1.10 ± 0.04 | 1.09 ± 0.05 |
| Mg ²⁺ (mM) | Smoking | 0.51 ± 0.04 | 0.51 ± 0.04 | 0.49 ± 0.04* | 0.48 ± 0.05* | 0.46 ± 0.05* |
| | Nonsmoking | 0.52 ± 0.04 | 0.52 ± 0.04 | 0.51 ± 0.04* | 0.49 ± 0.04* | 0.48 ± 0.05* |

Results are presented as mean ± SD.

* Statistically significant differences from elapsed time zero. There were no significant differences between the smoking and nonsmoking patients.

MAP = mean arterial pressure; T_{core} = core temperature.

increased over the study period in both groups, and the increase in aggregation was significantly greater in smoking than nonsmoking patients starting 2 h after induction of anesthesia (table 3).

The relative amount of mRNA for all cytokines was minimal at elapsed time zero. Gene expression of all cytokines except IL-6 increased significantly over time in both groups. Two hours after anesthesia, gene expression for all cytokines except IL-6 increased significantly in both groups. The increases in gene expression of IL-1 β , IFN- γ , and TNF- α were significantly smaller in the

smoking than the nonsmoking group. Between-group differences were not observed for IL-8. Gene expression for IL-6 did not increase over time in either group (fig. 1).

The fraction of alveolar macrophages ingesting opsonized and nonopsonized particles decreased significantly starting 2 and 4 h after anesthesia in the smoking and the nonsmoking groups, respectively. The decrease in both nonopsonized and opsonized phagocytosis in the smoking group reached 40–50% by the end of surgery, whereas the decrease in the nonsmoking group was 20–30% of baseline values. After 2 h of anesthesia, the

Table 3. Cell Recovery and Type from Bronchoalveolar Lavage

| | Time (h) | 0 | 2 | 4 | 6 | End |
|-------------------------------------|------------|----------|----------|----------|-----------|-----------|
| Recovery rate (%) | Smoking | 67 ± 9 | 65 ± 8 | 63 ± 6 | 64 ± 8 | 62 ± 9 |
| | Nonsmoking | 62 ± 8 | 66 ± 7 | 65 ± 6 | 67 ± 7 | 64 ± 8 |
| Cells ($\times 10^4/\text{cm}^3$) | Smoking | 63 ± 21† | 62 ± 21† | 65 ± 23† | 69 ± 24*† | 70 ± 26*† |
| | Nonsmoking | 15 ± 5 | 15 ± 4 | 15 ± 4 | 16 ± 5 | 16 ± 6 |
| Macrophage (%) | Smoking | 93 ± 3† | 90 ± 4* | 86 ± 4* | 83 ± 5* | 80 ± 8* |
| | Nonsmoking | 90 ± 3 | 90 ± 3* | 88 ± 4* | 86 ± 5* | 83 ± 7* |
| Lymphocyte (%) | Smoking | 6 ± 3† | 7 ± 3 | 8 ± 3 | 8 ± 2 | 8 ± 4 |
| | Nonsmoking | 9 ± 3 | 8 ± 3 | 8 ± 3 | 7 ± 4 | 8 ± 5 |
| Neutrophils (%) | Smoking | 1 ± 1 | 3 ± 3* | 6 ± 2* | 9 ± 5* | 12 ± 8* |
| | Nonsmoking | 1 ± 1 | 2 ± 2 | 4 ± 2* | 7 ± 4* | 9 ± 4* |
| Viability (%) | Smoking | 92 ± 4 | 93 ± 3 | 92 ± 4 | 92 ± 4 | 92 ± 5 |
| | Nonsmoking | 94 ± 3 | 95 ± 3 | 94 ± 3 | 94 ± 3 | 94 ± 2 |
| Aggregation (%) | Smoking | 6 ± 4 | 7 ± 4*† | 14 ± 5*† | 21 ± 5*† | 27 ± 7*† |
| | Nonsmoking | 4 ± 3 | 4 ± 4 | 8 ± 4* | 12 ± 5* | 17 ± 6* |

Data expressed as mean ± SD.

* Statistically significant differences from elapsed time zero.

† Significant differences ($P < 0.01$) between the smoking and nonsmoking groups.

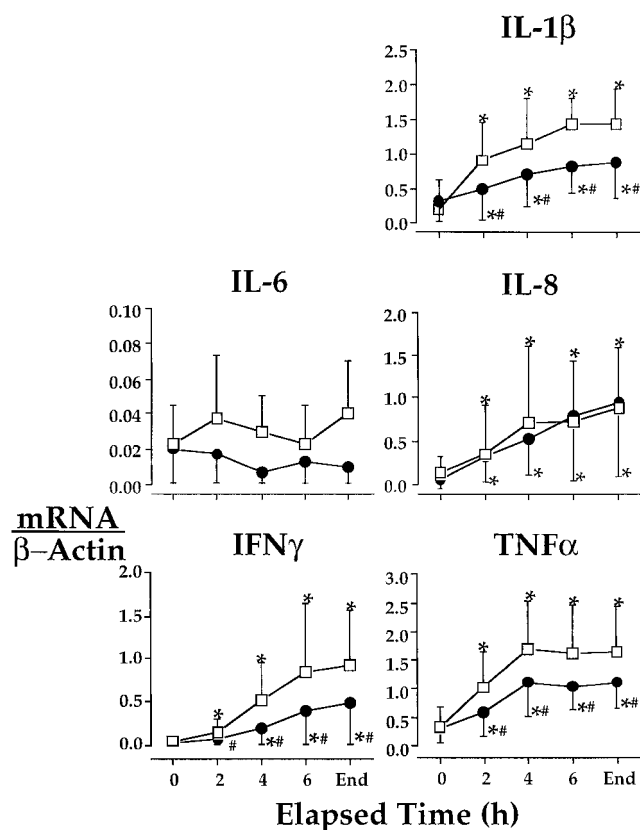


Fig. 1. Expression of proinflammatory cytokines during anesthesia in smoking (n = 30; circles) and nonsmoking (n = 30; squares) patients. Ratios of cytokine mRNA to β -actin mRNA are presented as the mean \pm SD. *Statistically significant difference ($P < 0.05$) from elapsed time zero in each group. #Statistically significant difference ($P < 0.01$) from the nonsmoking group.

decrease in phagocytosis was significantly greater in the smoking than in the nonsmoking patients (fig. 2).

Microbicidal activity, as evaluated by killing of *Listeria monocytogenes* at both 30 and 120 min postincubation, decreased significantly starting 2 and 4 h after anesthesia in the smoking and the nonsmoking groups, respectively. By the end of surgery, microbicidal activity decreased about 40% in the smoking patients; it decreased only about 20% in nonsmoking patients. Differences between the two groups were statistically significant starting 2 h after anesthesia (fig. 3).

Discussion

Expression of Proinflammatory Cytokines

Interleukin 1 β and TNF α are important proinflammatory cytokines in macrophages and neutrophils.⁹ Anes-

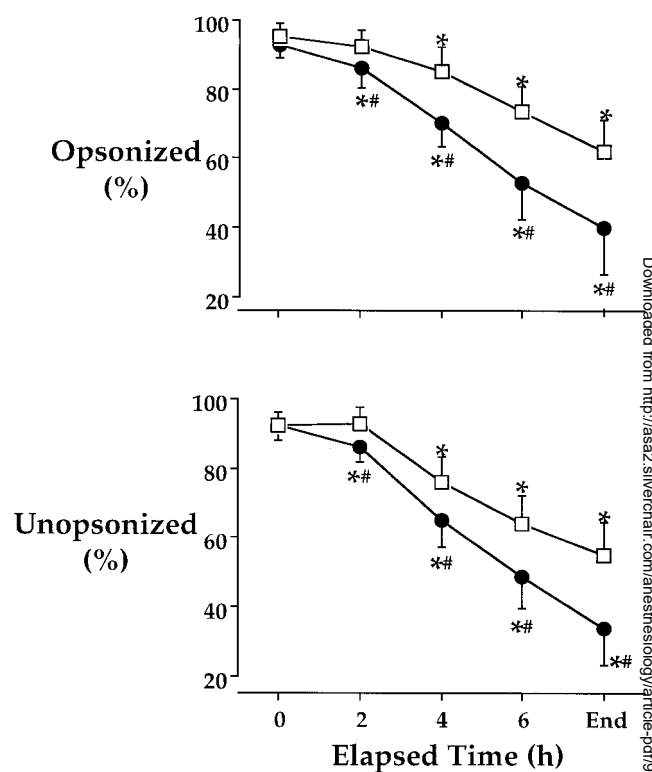


Fig. 2. Fraction of alveolar macrophages ingesting opsonized and nonopsonized particles in smoking (n = 30; circles) and nonsmoking (n = 30; squares) patients. Data are expressed as the mean \pm SD. *Statistically significant difference ($P < 0.05$) from elapsed time zero in each group. #Statistically significant difference ($P < 0.01$) from the nonsmoking group.

thesia and surgery augmented gene expression of IL-1 and TNF α , as well as IL-8 and IFN γ , in alveolar immune cells in our human³ and animal⁴ studies. Expression of these cytokines in alveolar immune cells, with the exception of IL-8, was reduced significantly in the smoking patients. Our results are also consistent with a previous animal study indicating that the increases in proinflammatory cytokine production and gene expression during halothane and isoflurane anesthesia were much smaller in smoke-exposed than in control rats.⁷

Gene expression for proinflammatory cytokines increases after pulmonary insults such as instillation of lipopolysaccharide or bacteria.^{10,11} Although acute and massive secretion of IL-1 and TNF α causes critical respiratory failure, IL-1 and TNF α potentiate immune resistance in wound healing and bacterial infection if secreted in controlled quantities.^{12,13} IFN γ is an immunostimulative cytokine to augment cell-mediated immune defense against bacterial insults in the macrophage-monocyte-neutrophil system. Reduced expression

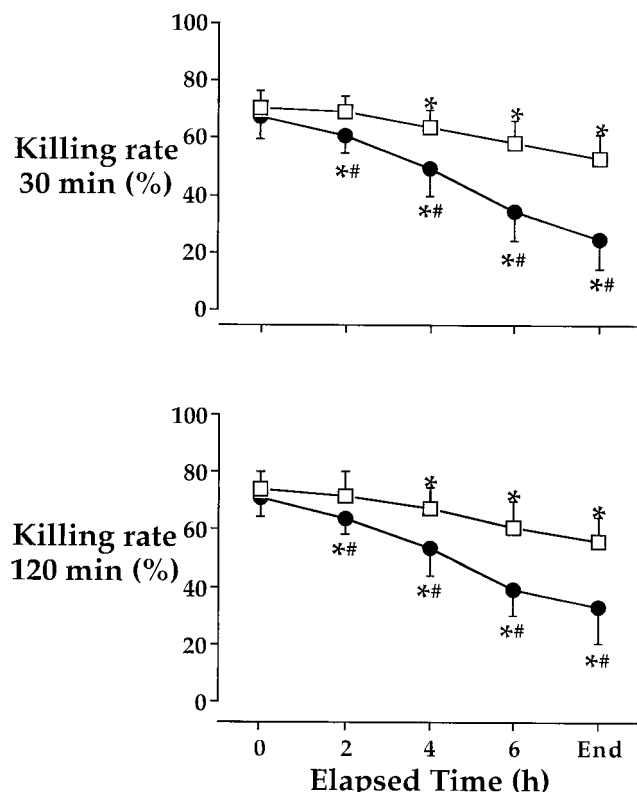


Fig. 3. The percentage of *Listeria monocytogenes* killed by alveolar macrophage from smoking ($n = 30$, circles) and nonsmoking ($n = 30$, squares) patients after 30 and 120 min incubation. Data are expressed as the mean \pm SD. *Statistically significant difference ($P < 0.05$) from elapsed time zero in each group; #statistically significant difference ($P < 0.01$) from the non-smoking group.

of these cytokines in smoking patients thus may impair pulmonary defenses during anesthesia and surgery.⁹

Interleukin 8 is among the most potent chemoattractants for neutrophils in humans. Expression of the IL-8 gene was comparable in smoking and nonsmoking patients. This finding is consistent with a previous study in unanesthetized patients.¹⁴ In rats, macrophage inflammatory protein 2 is a potent chemoattractant, as is IL-8. In our previous study, we found the expression and production of macrophage inflammatory protein 2 to be the same in smoke-exposed and control rats.⁷

We detected little or no expression of the gene for IL-6 in alveolar macrophages. This finding was comparable with previous studies.^{3,4,7} Why this gene is expressed so poorly during anesthesia and surgery remains unknown. McCrea *et al.*¹⁴ report that IL-6 was undetectable in bronchoalveolar lavage fluid from 50% of patients; however, they found that IL-6 concentrations were greater in smokers than nonsmokers. IL-6 is a pleiotropic cytokine

released by alveolar macrophages. Like IL-1 β and TNF- α , IL-6 is involved in acute-phase protein production and is an endogenous inflammatory mediator. Although alveolar macrophages can produce IL-6 *in vitro* and the production is more pronounced under stimulation of lipopolysaccharide, other cells such as alveolar epithelial cells or fibroblast may be the main sources *in vivo*.

Phagocytic and Microbicidal Activities

The effect of smoking on phagocytic and microbicidal activities is an area of controversy.¹⁵ Martin¹⁶ pointed out that different techniques of measurement lead to different conclusions. In our study design, preoperative phagocytic and microbicidal activities were similar in smoking and nonsmoking patients. Phagocytic and microbicidal activities are up-regulated by proinflammatory cytokines and growth factors, some of which (IL-1, TNF- α , IFN- γ) increase during anesthesia and surgery.^{13,17,18} Nonetheless, phagocytic and microbicidal activities decreased over time in both groups. These results were consistent with those we reported previously.² Similar reductions in microbicidal activity of alveolar macrophages have been reported to occur with exposure to volatile anesthetics.^{19,20} It is well established that antimicrobial functions of other immune cells including monocytes and neutrophils decrease during anesthesia and surgery.^{21,22} In contrast, expression and concentrations of proinflammatory cytokines increase during anesthesia.²³ Volatile anesthetics markedly suppress the production of adenosine triphosphate and protein in alveolar macrophages.^{24,25} We found that cytokine expression does not always correlate with cytokine production.^{4,7} The decrease in metabolic activity by anesthetics may explain this observation partially.

A notable finding of this study is that phagocytic and microbicidal activities decreased almost twice as much in smoking as nonsmoking patients. There are numerous potential explanations for this observation:

1. Decreased activation of alveolar macrophages in smokers may result from reduced expression of proinflammatory cytokines.
2. Decreased phagocytic and microbicidal activities are consistent with the observation that metabolic activity in human alveolar macrophages is reduced in patients who smoke.²⁶
3. Smoking-induced inhibition of metabolic activity in alveolar macrophages is compounded by anesthesia,²⁵ resulting in a further decrease in phagocytic and microbicidal activities.

4. Smoking decreases the normal phagocytosis-induced activation of alveolar macrophages.²⁷
5. Macrophage aggregation was much greater in smoking than nonsmoking patients. At the very least, aggregation decreases cell membrane surface area available for phagocytosis, thus reducing the ability of alveolar macrophages to ingest foreign particles.

Phagocytosis and bactericidal activity of alveolar macrophages are key elements of pulmonary defense. Smoking markedly reduced both functions, suggesting that smokers have reduced ability to resist pulmonary bacterial infection during anesthesia and surgery. This observation is consistent with the observation that postoperative pulmonary complications are far more common in smokers than nonsmokers.^{5,6} Macrophage aggregation and neutrophil influx were observed after 2 and 4 h of anesthesia in smoking and nonsmoking patients, respectively. There were no significant differences, however, in neutrophil number between two groups. Our results thus suggest that at the very least, neutrophil chemotactic activity is not suppressed in smokers.^{28,29} This observation is consistent with our observation that expression of IL-8 was similar in the two groups.

Aggregation of alveolar macrophages was increased markedly in smoking patients. Hegab and Matulionis³⁰ reported that anesthesia causes massive macrophage aggregation in smoke-exposed mice, whereas this response was absent in unexposed mice. Adhesion molecules are a critical component of macrophage and neutrophil accumulation. Schaberg *et al.*^{31,32} reported that alveolar macrophages and pulmonary vascular epithelial cells from smokers demonstrated augmented expression of CD11a/CD18, CD11b/CD18, CD11c/CD18, and CD54 (intercellular adhesion molecule 1), all of which are leukocyte adhesion molecules. The accumulated and activated neutrophils up-regulate local adhesion molecules.³³ Klut *et al.*²⁸ reported that neutrophils within pulmonary microvessels are activated by smoke-exposed rabbits. Various factors contributing to macrophage aggregation, such as Mg^{2+} , Ca^{2+} , and body temperature, were comparable in each group.³⁴ Taken together, these results suggest that preoperative increases in adhesion molecules lead to subsequent accumulation of inflammatory cells to the distal airway and macrophage aggregation in smoking patients.

We found an increase in the number of alveolar immune cells from smoking patients after 4 h of anesthesia. A study by Hegab and Matulionis³⁰ showed that smoke exposure in mice prompts migration of parenchymal

macrophages to the distal airway. They speculated that some pulmonary cells liberate chemotactic substances that attract parenchymal immune cells, a conclusion supported by the work of Koyama *et al.*²⁹ Accumulation of alveolar immune cells in the smoking patients thus may result in part because smoking provoked migration of parenchymal and vascular cells to the distal airway.

Another possible explanation for the increased number of alveolar immune cells in the smokers is impairment of mucociliary transport by smoking and anesthesia. Macrophages are removed from the alveoli in part by the mucociliary transport system; impairment of this system results in alveolar macrophage accumulation in the distal airway. This mechanism is seriously impaired by ciliotoxins of cigarettes such as hydrocyanic acid, acetaldehyde, acrolein, and formaldehyde.⁵ Anesthesia also damages mucociliary transport^{35,36} and is likely to facilitate accumulation of alveolar immune cells synergistically in the airways of smokers.

Limitation and Summary

A limitation of our study is that we did not measure the concentration of proinflammatory cytokines in bronchoalveolar lavage fluid. This was in part because there is no way of estimating the volume of the epithelial lining fluid, making it impossible to quantify the concentration of cell products in the bronchoalveolar lavage fluid. Although concentrations of potassium, protein, and urea have been used as markers, these provide only a rough index of dilution. Estimation of cytokine concentrations would have been especially problematic given the observed large time-dependent changes in alveolar macrophage number, aggregation, and activity. Also, most cytokines may be secreted in autocrine and paracrine modes. Hence, we directly evaluated inflammatory responses at transcriptional levels.

To avoid any artificial influence of the sorting process we did not separate the macrophages from other cells. For example, even adherence to plastic test tubes for separation of alveolar macrophage activates gene expression of cytokines.³⁷ Although macrophages are the most likely source in initial responses, IFN- γ is a potent stimulator of alveolar macrophages; this cytokine, however, is mainly secreted by activated lymphocytes. IL-8 is expressed and secreted from alveolar macrophages to recruit neutrophils. Furthermore, migrated and activated neutrophils produce lysosomal enzymes and oxygen free radicals, which facilitate IL-8 secretion in the lungs significantly.^{38,39} Thus, we cannot draw a definite conclu-

sion regarding cell source for the observed increase in gene transcription.

We conclude that the antimicrobial and proinflammatory responses of alveolar macrophages during anesthesia and surgery differ markedly in smokers and nonsmokers. Antimicrobial and proinflammatory responses of alveolar immune cells, at both transcriptional and histologic levels, are suppressed markedly in smokers.

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