Recovery of Intraoperative Microbicidal and Inflammatory Functions of Alveolar Immune Cells after a Tobacco Smoke–free Period

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Background: Tobacco smoking inhibits alveolar macrophage function, but cessation of smoking markedly reduces the risk of postoperative pulmonary complications. The authors therefore evaluated the effect of nonsmoking duration on both antimicrobial and inflammatory functions of alveolar macrophages during anesthesia and surgery.

Methods: The authors studied 15 patients who had never smoked, 15 current smokers, and 41 former smokers, all of whom underwent general anesthesia. Former smokers were further allocated to one of three groups depending on their smoke-free periods: 2 months (n = 13), 3–5 months (n = 13), and 6–12 months (n = 15). Alveolar immune cells were collected by bronchoalveolar lavage immediately after induction of anesthesia, at 2 and 4 h after induction of anesthesia, and at the end of surgery. Opsonized and nonopsonized phagocytosis were measured. Microbicidal activity was determined as the ability of the macrophages to kill Listeria monocytogenes directly. Finally, we determined the expression of proinflammatory cytokines, including interleukin 1ß, interleukin 8, inter-feron γ, and tumor necrosis factor α, and of antiinflammatory cytokines (interleukin 4 and 10) by semiquantitative polymerase chain reaction.

Results: Nonopsonized and opsonized phagocytosis and microbicidal activity of alveolar macrophages (antimicrobial functions) decreased 20–50%, and the expression of genes for all proinflammatory and antiinflammatory cytokines increased 3–30-fold over time in all groups. Starting 4 h after induction of anesthesia, the decreases in antimicrobial functions were 1.5–5 times greater in current and former smokers (2 months’ abstinence) than in patients who had never smoked. Starting 4 h after anesthesia, the increase in expression of all cytokines, except interleukin 8, was twofold to fivelfold less in current and former smokers (2–6 months’ abstinence) than in patients who had never smoked.

Conclusion: Our data suggest that former smokers may have a limited ability to mount effective pulmonary immune defenses for long as 6 months after stopping cigarette use.

ALVEOLAR immune cells, which are 90% macrophages, are the first line of pulmonary defense. One of their major functions is antimicrobial activity, especially phagocytic and microbicidal actions against foreign invaders. Alveolar immune cells also have proinflammatory and antiinflammatory functions and attract neutrophils to the distal airway. We previously reported a significant time-dependent decrease in antimicrobial functions, including phagocytosis and microbicidal activity, of alveolar macrophages during anesthesia and surgery.1–5 In contrast, macrophage aggregation and neutrophil influx are augmented during anesthesia and surgery.1–5 Smoking also seriously impairs antimicrobial10,11 and proinflammatory12,13 functions. Proinflammatory and antimicrobial functions during anesthesia and surgery are thus further modulated by preoperative smoking. As a result, both antimicrobial and proinflammatory functions decrease substantially more during halothane and isoflurane anesthesia in smoke-exposed rats than in unexposed animals.5 We similarly found that patients who were currently smokers had impaired antimicrobial and proinflammatory alveolar macrophage functions during anesthesia and surgery.4 These data are consistent with the clinical observation that postoperative pulmonary complications are up to five times more common in smokers than in nonsmokers.14,15 It is likely that many of these complications are mediated by impaired alveolar macrophage function.1–9

Even short smoke-free intervals are thought to benefit surgical patients by eliminating carboxyhemoglobin, improving ciliary function, and decreasing both mucus secretion and small airway obstruction. According to Bluman et al.15 a 2-month smoke-free intervals before surgery reduces the risk of postoperative complications to the level observed in nonsmokers. At the same time, they reported that cessation of smoking for only 1 month is not associated with a decreased risk of postoperative pulmonary complications. These results suggest that resistance to pulmonary complications remains poor for a considerable period after smoking cessation.

We thus characterized the tobacco smoke-free interval required to normalize activity of alveolar macrophages during anesthesia and surgery. As in our previous
studies,1–7 we evaluated a number of alveolar macrophage functions, including (1) opsonized and nonopsonized phagocytosis; (2) microbicidal activity; (3) macrophage aggregation; (4) neutrophil influx to the distal airway; and (5) expression of genes for proinflammatory cytokines, including interleukin 1β, interleukin 8, interferon γ, and tumor necrosis factor α (TNF-α). We also took this opportunity to measure, for the first time, the effect of tobacco smoke exposure on expression of genes for the antiinflammatory cytokines interleukin 4 and 10.

Methods

The protocol for this study was approved by the Institutional Review Board of the University of Hirosaki (Hirosaki, Japan), and written informed consent was obtained from all participating patients. We studied 15 patients who had never smoked, 15 current smokers, and 41 former smokers who were scheduled to undergo general anesthesia for more than 4 h. Former smokers were further allocated to one of three groups, depending on their smoke-free periods: 2 months (n = 13), 3–5 months (n = 13), and 6–12 months (n = 15). Identifying suitable patients who had quit smoking within the year before surgery was difficult. Consequently, former smokers were enrolled over a 6-yr period, from 1994 to 2000. Nonsmokers were studied during the same period.

We excluded patients with one or more of the following conditions: (1) chronic obstructive or restrictive pulmonary disease; (2) American Society of Anesthesiologists physical status greater than II; (3) current steroidal or nonsteroidal antiinflammatory medications; (4) pulmonary or other infection or abnormal chest radiograph; (5) neoplastic disease; (6) forced vital capacity and pulmonary function tests less than 80% of predicted; and (7) body mass index greater than 30.

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 maintained with propofol (5–8 mg · kg⁻¹ · h⁻¹), fentanyl (10–20 μg/kg), and vecuronium. A volume-controlled ventilator was set to 10 ml/kg; the respiratory rate was then adjusted to produce an arterial carbon dioxide partial pressure between 35 and 45 mmHg. The inspiratory/expiratory ratio was 0.5, and positive end-expiratory pressure was not used. Radial arterial pressure, electrocardiogram, pulse oximeter saturation, and the bispectral index were monitored in all patients. A catheter was inserted via the right internal jugular vein to monitor central venous pressure.

All patients were transported to the recovery unit immediately after surgery. After the trachea was extubated, the patients were given 40% oxygen via a nonrebreathing mask. Supplemental oxygen was given to patients in all groups as necessary to maintain pulse oximeter saturation of at least 95%.

Evaluation of Pulmonary Function and Complications

Arterial blood was sampled for gas analysis before each bronchoalveolar lavage and 1 h after extubation while breathing 40% oxygen. The saturation from a pulse oximeter, end-tidal carbon dioxide partial pressure, and peak airway pressure were measured at 30-min intervals during anesthesia. Saturation and blood pressure were recorded every 30 min during recovery.

Postoperative pulmonary complications, as previously described,4–5 were evaluated by a physician who was unaware of the patients’ group assignment and intraoperative management. The anesthesiologists providing intraoperative care, physicians performing bronchoalveolar lavage, and the investigators measuring various biochemical mediators were fully blinded to the group assignments.

Bronchoalveolar Lavage

Bronchoalveolar lavage was based on our previously described methodology. The lungs were lavaged immediately after induction of anesthesia, 2 and 4 h after induction of anesthesia, and at the end of surgery.2,4,5,7 Briefly, a bronchovideoscope 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 instillation of 20 ml of sterile balanced saline solution, as reported previously.2,4,5,7 Total instillation of solution was 100 ml. A different randomly chosen segment was lavaged each time, and the same investigator performed all the bronchoalveolar lavages.

After straining through a single layer of loose cotton gauze for removal of mucus, we counted the number of alveolar macrophages and the viability of alveolar cells. Cell differentiation and aggregation were examined as previously described in detail.1–5,7

Expression of Proinflammatory and Antinflammatory Cytokine Genes

The following molecular analysis of proinflammatory cytokines was based on our previously reported method.3–7 However, this was modified by our use of glyceraldehyde-3 phosphate dehydrogenase (G3PDH) as an internal standard. Briefly, the cell pellets were dissolved immediately in 0.5 ml of guanidinium buffer solution.3–8 RNA was isolated by the well-established acid guanidinium-phenol-chloroform method.3–8 We obtained 2.4–5.9 μg of RNA from each sample; cDNA was syn-
thetised at 40°C for 60 min from 0.25 μg of RNA by reverse transcription.

The semiquantitative reverse-transcription polymerase chain reaction (PCR) mixture (50 μl) contained cDNA synthesized from 0.2 μg RNA, Tris-HCl (pH 8.3), potassium and magnesium, deoxyribonucleic triphosphate, oligonucleotide primers, and a DNA polymerase. The reaction mixture was then amplified. Each cycle consisted of denaturation at 94°C for 1 min, annealing at 56°C (interleukin-6 and interferon 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 conducted during the exponential phase of amplification. The number of PCR cycles for each primer set was as follows: 32 for interferon γ, 29 for interleukin 18, and 27 for interleukin 4, interleukin 8, interleukin 10, TNF-α, and G3PDH. The sequences of sense and antisense primers of G3PDH, interleukin 4, and interleukin 10 are shown in Table 1. Primer pairs for the other cytokines were reported previously. The optimal number of PCR cycles for each primer set was determined in preliminary experiments so that the amplification process was conducted during the exponential phase of amplification. The number of PCR cycles for each primer set was as follows: 32 for interferon γ, 29 for interleukin 18, and 27 for interleukin 4, interleukin 8, interleukin 10, TNF-α, and G3PDH. The sequences of sense and antisense primers of G3PDH, interleukin 4, and interleukin 10 are shown in Table 1. Primer pairs for the other cytokines were reported previously. The G3PDH 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 G3PDH.

The PCR products were separated by electrophoresis on a 1.8% agarose gel containing 0.5 g/ml ethidium bromide, with the products visualized and quantified as described previously. We calculated the cytokine/G3PDH ratio of the intensity of ethidium bromide fluorescence for each PCR product.

### Phagocytic and Microbicidal Activities

Phagocytic and microbicidal activities were evaluated as previously described. Briefly, alveolar macrophages were resuspended in a balanced saline solution supplemented with calcium and magnesium. Nonopsonized and opsonized (1.0-μm diameter) particles were added, with a particle-to-cell ratio of 15:1. The cell suspension was placed on a glass slide, fixed, and stained. We recorded the fraction of cells that ingested at least one particle and the number of fluorescent particles in each.

The bactericidal capacity of the alveolar macrophages was determined by their ability to kill *Listeria monocytogenes* according to our previously described method. Alveolar macrophages were separated and resuspended in RPMI-1640 containing 10% normal human serum. *L. monocytogenes* were resuspended, mixed with the alveolar macrophages, and incubated for 30 and 120 min. Centrifuged pellets of alveolar macrophages were lysed for release of bacteria. The viable fraction of *Listeria* bacteria was determined by plating of serial 10-fold dilutions on agar plates. The number of colonies of *Listeria* was counted after 48 h on one of the plates. We calculated the rate at which alveolar macrophages killed *Listeria*.

### Data Analysis

The period immediately after induction of anesthesia was designated as elapsed time zero. Time-dependent intragroup data were evaluated by repeated analysis of variance and post hoc Dunnett tests for comparison to elapsed time zero. Differences among groups at each time point were evaluated using the chi-square test or factorial analysis of variance, followed by a post hoc Dunnett test. P < 0.01 was considered statistically significant. Data are expressed as mean ± SD.

### Results

Age, sex, American Society of Anesthesiologists physical status, body weight, forced vital capacity, forced expiratory volume in 1 s, anesthesia time, and total dose of fentanyl did not differ significantly among the groups (data not shown). Table 2 shows that initial intraoperative cardiorespiratory measurements, esophageal temperature, bispectral index, and plasma electrolytes were similar in each of the five groups, as were time-dependent changes. Intraoperative blood loss was less than 500 ml in all patients, and no blood or blood products were transfused. Nonsmoking patients did not experience postoperative pulmonary complications. However, one current smoker and one former smoker developed mild postoperative pulmonary infections.

The percentage of macrophages at elapsed time zero was slightly but significantly greater in the smokers and those with 2 months of abstinence than in the nonsmokers. The percentage of lymphocytes at this point was less in the smoking and 2-month smoke-free patients than in the nonsmoking patients. The percentage of neutrophils increased significantly as a function of anesthetic duration, whereas the percentage of macrophages decreased in all groups. There were no differences in the percentages of each cell type among the groups at 4 h after anesthesia or at the end of surgery (Table 3).

There were no statistically significant differences in the rate of recovery of bronchoalveolar lavage fluid as a function of time within or among groups. However, the concentrations and total numbers of alveolar cells were four to five times greater in smoking than in nonsmoking patients at all times. In patients who had abstained from
smoking for 2 months, the concentrations and total number of alveolar cells in lavage fluid decreased to one half of those in smoking patients. However, the values were still greater in those who had abstained for 3–5 months than in nonsmokers. The total numbers of cells in the smokers and those who had quit 2 months before surgery both increased by the end of surgery. The fraction of aggregated cells increased 2 or 4 h after anesthesia in all groups. The increase in the fraction of aggregated cells was significantly greater in current smokers and in those who had been smoke-free for 3–5 months than in nonsmoking patients starting 2 h after anesthesia (fig. 1).

Gene expression for each proinflammatory cytokine was minimal at elapsed time zero in all groups. Gene expression of the proinflammatory cytokines increased significantly in all groups within 2 or 4 h after induction of anesthesia. Starting 4 h after anesthesia, the increases in gene expression of interleukin 1β, interferon γ, and TNF-α were significantly less in the smokers and those who had quit less than 6 months before surgery than in nonsmokers (fig. 2).

Gene expression for antiinflammatory cytokines was also minimal at elapsed time zero in all groups, but expression of interleukin 4 and 10 increased starting 4 h after induction of anesthesia. Expression of interleukin 10 increased more than 30-fold, whereas interleukin 4 expression only tripled (data not shown). However, these increases were significantly less in smokers and those who had quit for less than 6 months than in nonsmokers (fig. 3).

The fraction of alveolar macrophages ingesting opsonized and nonopsonized particles and bactericidal activity as evaluated by killing of L. monocytogenes at both 30 and 120 min after incubation were similar in each of the five groups at elapsed time zero. Each of these values decreased significantly starting 2 or 4 h after anesthesia in all groups. However, again, starting at 4 h of anesthesia, the decreases in both phagocytosis and bactericidal activity were significantly greater in smokers and those who had quit for only 2 months than in nonsmokers (figs. 4 and 5).

### Discussion

**Expression of Proinflammatory Cytokines**

We have reported that the time-dependent increases in expression of cytokines, with the exception of interleukin 8 in humans and macrophage inflammatory protein 2 in rats, were significantly smaller in the smoking than in nonsmokers (fig. 1).

### Table 2. Intraoperative Data

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Mean arterial pressure (mmHg)</th>
<th>Heart rate (beats/min)</th>
<th>pH</th>
<th>PaCO₂ (mmHg)</th>
<th>PaO₂ (mmHg)</th>
<th>Peak inspiratory pressure (cm H₂O)</th>
<th>Esophageal temperature (°C)</th>
<th>Ionized calcium (mM)</th>
<th>Ionized magnesium (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>76 ± 9</td>
<td>70 ± 11</td>
<td>7.42 ± 0.03</td>
<td>132 ± 24</td>
<td>15.8 ± 2.3</td>
<td>36.7 ± 0.4</td>
<td>0.1 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>92 ± 13*</td>
<td>81 ± 12*</td>
<td>7.38 ± 0.03*</td>
<td>132 ± 22</td>
<td>15.7 ± 2.2</td>
<td>36.3 ± 0.5*</td>
<td>1.1 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>End</td>
<td>93 ± 11*</td>
<td>81 ± 12*</td>
<td>7.36 ± 0.04*</td>
<td>132 ± 23</td>
<td>15.7 ± 1.9</td>
<td>36.3 ± 0.5*</td>
<td>1.1 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

Results are mean ± SD.

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

**Table 3. Distribution of Alveolar cells**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Nonsmokers</th>
<th>Former Smokers (6–12)</th>
<th>Former Smokers (3–5)</th>
<th>Former smokers (2)</th>
<th>Current Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>90.4 ± 2.7</td>
<td>91.1 ± 3.9</td>
<td>91.0 ± 3.6</td>
<td>92.9 ± 2.8*</td>
<td>94.4 ± 2.5*</td>
</tr>
<tr>
<td>4</td>
<td>87.1 ± 4.7†</td>
<td>88.3 ± 3.8†</td>
<td>86.5 ± 4.5†</td>
<td>86.1 ± 4.1†</td>
<td>86.8 ± 4.3†</td>
</tr>
<tr>
<td>End</td>
<td>85.7 ± 4.5†</td>
<td>85.3 ± 5.5†</td>
<td>83.3 ± 4.7†</td>
<td>83.4 ± 4.4</td>
<td>82.7 ± 6.0†</td>
</tr>
<tr>
<td>0</td>
<td>8.2 ± 2.2</td>
<td>7.6 ± 3.0</td>
<td>7.5 ± 3.5</td>
<td>5.5 ± 2.6*</td>
<td>4.3 ± 2.5*</td>
</tr>
<tr>
<td>4</td>
<td>7.1 ± 3.1</td>
<td>6.6 ± 3.1</td>
<td>5.7 ± 2.8</td>
<td>5.6 ± 4</td>
<td>4.8 ± 1.9</td>
</tr>
<tr>
<td>End</td>
<td>7.2 ± 3.8</td>
<td>6.5 ± 3.5</td>
<td>7.4 ± 2.9</td>
<td>6.2 ± 3.0</td>
<td>5.6 ± 3.0</td>
</tr>
<tr>
<td>0</td>
<td>1.4 ± 1.3</td>
<td>1.3 ± 1.2</td>
<td>1.6 ± 1.2</td>
<td>1.6 ± 1.1</td>
<td>1.3 ± 1.7</td>
</tr>
<tr>
<td>4</td>
<td>5.7 ± 3.8†</td>
<td>5.1 ± 2.8†</td>
<td>7.9 ± 4.4†</td>
<td>8.3 ± 3.3†</td>
<td>8.4 ± 4.6†</td>
</tr>
<tr>
<td>End</td>
<td>7.0 ± 4.4†</td>
<td>8.2 ± 5.2†</td>
<td>9.3 ± 2.7†</td>
<td>10.4 ± 3.5†</td>
<td>11.8 ± 5.8†</td>
</tr>
</tbody>
</table>

Nonsmoking duration is in months. Results are mean ± SD.

* Statistically significant differences ($P < 0.05$) from nonsmokers. † Statistically significant differences ($P < 0.05$) from elapsed time 0.
nonsmoking subjects during anesthesia and surgery.\textsuperscript{3,4}

Our current results in patients who continued to smoke confirm our previous results. These results are also consistent with previous nonanesthesia studies, which showed that the \textit{in vitro} production of proinflammatory cytokines in alveolar macrophages after pulmonary insult were significantly smaller in smoking than in non-smoking patients.\textsuperscript{12,13}

One of our important findings is that it takes at least 6 months for proinflammatory functions to normalize at the transcriptional level in alveolar macrophages during anesthesia and surgery.\textsuperscript{5,4}

Our current results in patients who continued to smoke confirm our previous results. These results are also consistent with previous nonanesthesia studies, which showed that the \textit{in vitro} production of proinflammatory cytokines in alveolar macrophages after pulmonary insult were significantly smaller in smoking than in non-smoking patients.\textsuperscript{12,13}

One of our important findings is that it takes at least 6 months for proinflammatory functions to normalize at the transcriptional level in alveolar macrophages during anesthesia and surgery. The normalization in the concentration and total number of alveolar macrophages was observed after a smoke-free period of approximately 6 months, a time that far exceeds the 80-day median life length of the alveolar macrophages. These findings suggest that smoking impairs cell replication for a prolonged period of time. In fact, this theory is supported by the work of Sherman \textit{et al.}\textsuperscript{16} who showed that smoking damages DNA in human alveolar macrophages. Furthermore, Sköld \textit{et al.}\textsuperscript{17} have shown that tobacco-related fluorescent particles can be detected in alveolar macrophages by flow cytofluorometry after more than 6 months of abstinence. Taken together, these studies indicate that the inflammatory functions of alveolar macrophages remain impaired for at least 6 months after cessation of smoking.

The clinical significance of increased gene expression of interleukin 1\(\beta\) and TNF-\(\alpha\) remains unclear, because these cytokines can potentiate immune defenses\textsuperscript{18,19}—or cause respiratory failure.\textsuperscript{20} However, interferon \(\gamma\) is an immunosimulative cytokine that augments cell-mediated immune defense against bacterial insults in the macrophage-monocyte-neutrophil system. Reduced expression of these cytokines suggests that intraoperative proinflammatory functions of alveolar macrophages remain suppressed even after a 6-month tobacco smoke-free period.

\textbf{Expression of Antiinflammatory Cytokines}

Increased concentrations of proinflammatory cytokines are usually followed by increases in antiinflammatory cytokines.\textsuperscript{21} For example, both plasma proinflammatory and antiinflammatory responses increase during general surgery and cardiopulmonary bypass.\textsuperscript{22,23} We observed a similar pattern at the transcriptional levels in alveolar immune cells: expression of genes for most proinflammatory cytokines increased after 2 h of anesthesia, whereas the compensatory increase in expression of antiinflammatory cytokines was delayed an additional 2 h. Kawamura \textit{et al.}\textsuperscript{23} reported marked increases in plasma interleukin 10 without concomitant changes in plasma interleukin 10 without concomitant changes.

\textbf{Expression of Proinflammatory Cytokines (interleukin [IL] 1\(\beta\), interleukin 8, interferon [IFN] \(\gamma\), and tumor necrosis factor [TNF]-\(\alpha\))}

Fig. 2. Expression of proinflammatory cytokines (interleukin [IL] 1\(\beta\), interleukin 8, interferon [IFN] \(\gamma\), and tumor necrosis factor [TNF]-\(\alpha\)) 4 h after induction of anesthesia (open bars) and at the end of surgery (hatched bars) in nonsmokers (NS, \(n = 15\)), former smokers not smoking for 6–12 months (6–12, \(n = 15\)), for 3–5 months (3–5, \(n = 13\)), for 2 months (2, \(n = 13\)), and current smokers (S, \(n = 15\)). *Statistically significant differences (\(P < 0.01\)) from the nonsmokers at each time point. Data are expressed as mean \(\pm\) SD.

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\(\text{mRNA} / \text{G3PDH}\)

\begin{align*}
\text{IL-1\(\beta\)} & \quad 0.5 & \quad 1 & \quad 2 & \quad 3 \\
\text{IFN\(\gamma\)} & \quad 0.5 & \quad 1 & \quad 2 & \quad 3 \\
\text{IL-8} & \quad 0.5 & \quad 1 & \quad 2 & \quad 3 \\
\text{TNF-\(\alpha\)} & \quad 0.5 & \quad 1 & \quad 2 & \quad 3 \\
\end{align*}

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\textbf{Expression of mRNA Levels in Alveolar Macrophages (IL-1\(\beta\), IL-8, IFN-\(\gamma\), and TNF-\(\alpha\))}

Fig. 2. Expression of proinflammatory cytokines (interleukin [IL] 1\(\beta\), interleukin 8, interferon [IFN] \(\gamma\), and tumor necrosis factor [TNF]-\(\alpha\)) 4 h after induction of anesthesia (open bars) and at the end of surgery (hatched bars) in nonsmokers (NS, \(n = 15\)), former smokers not smoking for 6–12 months (6–12, \(n = 15\)), for 3–5 months (3–5, \(n = 13\)), for 2 months (2, \(n = 13\)), and current smokers (S, \(n = 15\)). *Statistically significant differences (\(P < 0.01\)) from the nonsmokers at each time point. Data are expressed as mean \(\pm\) SD. mRNA = messenger RNA; G3PDH = glyceraldehyde-3-phosphate dehydrogenase.
in plasma interleukin 4 after cardiopulmonary bypass.

Their report is thus consistent with our observation that expression of interleukin 10 increased up to 30-fold, whereas expression of the gene for interleukin 4 increased only twofold to threefold. Which alveolar immune cells contributed the observed increases in gene expression of interleukin 4 and 10 remains unclear. Both interleukin 4 and 10 are mainly produced by activated T-helper type 2 lymphocytes. Typically, the ratio of plasma T-helper type 1 to type 2 cell subsets increases intraoperatively; the ratio may similarly have increased in alveolar lymphocytes. Alternatively, interleukin 4 and 10 may have been produced by alveolar macrophages that are activated during anesthesia and surgery.1–9

The effects of smoking on antiinflammatory cytokines of alveolar immune cells remains poorly described. Our novel finding is that intraoperative increases in gene expression for antiinflammatory cytokines were reduced far more in smokers than in nonsmoking patients. The decreases in interleukin 4 and 10 in smokers are likely to be important because both cytokines protect against various types of lung injury.26–28 Interleukin 10, for example, helps to protect against lung injury and improves survival from Pseudomonas aeruginosa pneumonia. Pulmonary infection induced by an intraoperative decrease in antimicrobial and proinflammatory responses may thus lead to further pulmonary injury by a decrease in antiinflammatory responses in smokers.

Phagocytic and Bactericidal Activities

The effect of smoking on phagocytic and microbicidal activities remains controversial.29 Martin11 pointed out that different measurement techniques lead to different conclusions. In the current study, preoperative phagocytic and microbicidal activities were similar in current smokers, former smokers, and nonsmoking patients. These results are consistent with our previous studies and a study by Jonsson et al. who evaluated direct phagocytic and killing of Haemophilus influenzae.

We have reported decreases in phagocytic and microbicidal activities during anesthesia and surgery.2–5 It seems likely that the marked intraoperative increase in antiinflammatory cytokines contribute to the observed reductions in phagocytic and bactericidal activity because interleukin 4 and 10 suppress proinflammatory cytokines that up-regulate phagocytic and bactericidal activity.19,33,34 Activation of other antiinflammatory responses such as adrenal hormones (by surgical stress) also suppress antimicrobial functions. For example, Dahanukar et al. reported that reduced intraoperative phagocytosis is inversely correlated with serum cortisol concentrations. Epinephrine similarly produces a dose-dependent inhibition of phagocytosis.36 Greater decreases in antiinflammatory responses in smokers than in nonsmokers seems consistent with reduced

Fig. 3. Expression of antiinflammatory cytokines (interleukin [IL] 4 and 10) 4 h after induction of anesthesia (open bars) and at the end of surgery (hatched bars) in nonsmokers (NS, n = 15), former smokers not smoking for 6–12 months (6–12, n = 15), for 3–5 months (3–5, n = 13), for 2 months (2, n = 13), and current smokers (S, n = 15). *Statistically significant differences (P < 0.01) from the nonsmokers at each time point. Data are expressed as mean ± SD. mRNA = messenger RNA; G3PDH = glyceraldehyde-3-phosphate dehydrogenase.

Fig. 4. The fraction of alveolar macrophages ingesting opsonized and nonopsonized particles 4 h after induction of anesthesia (open bars) and at the end of surgery (hatched bars) in nonsmokers (NS, n = 15), former smokers not smoking for 6–12 months (6–12, n = 15), for 3–5 months (3–5, n = 13), for 2 months (2, n = 13), and current smokers (S, n = 15). *Statistically significant differences (P < 0.01) from the nonsmokers at each time point. Data are expressed as mean ± SD.
Antimicrobial functions in smokers. However, proinflammatory responses were also markedly suppressed in the smokers. To the extent that antimicrobial functions are modulated by cytokines, these data suggest that antiinflammatory cytokines dominate in smokers.

There are a number of potential explanations for decreased intraoperative antimicrobial activity. Smoking markedly suppresses the metabolic activity of human alveolar macrophages, an inhibition that is compounded by anesthesia. Reduced metabolic activity, and therefore decreased phagocytic and microbial activity, impairs the ability of alveolar macrophages to respond to bacterial challenges. In contrast to the proinflammatory and antiinflammatory functions, phagocytic and bactericidal activities in alveolar macrophages were normal within 3 months of smoking cessation. This response may be a result, in part, of normalization of metabolic intensity, and indirect exposure to tobacco smoke and dust particles.

Mucociliary transport is seriously impaired by cilitoxins of tobacco such as hydrocyanic acid, acetaldehyde, acrolein, and formaldehyde. Anesthesia also damages mucociliary transport and is likely to synergistically facilitate accumulation of alveolar immune cells in the airways during anesthesia in current and recent former smokers. It thus seems likely that impaired ciliary transport explains the increased number of alveolar immune cells at the end of surgery in smokers and in those who had quit only 2 months before surgery.

**Limitations and Summary**

Cytokine expression does not necessarily correlate with cytokine production. We thus cannot determine whether the proinflammatory or antiinflammatory responses were comparable in our patients. We did not evaluate these responses at the protein levels, in part because there is no reliable way to estimate the volume of the epithelial-lining fluid, making it impossible to quantify the concentration of cell products in the bronchoalveolar lavage fluid. Estimation of cytokine concentrations would have been especially problematic given the observed large time-dependent changes in alveolar macrophage number, aggregation, and antimicrobial activity. It is further likely that most cytokines are secreted in autocrine and paracrine modes. Hence, we directly evaluated inflammatory responses at the transcriptional level.

We conclude that the antimicrobial and proinflammatory and antiinflammatory responses of alveolar macrophages during anesthesia and surgery were markedly suppressed in current and recent former smokers at both
the transcriptional and morphologic levels. Our results suggest that former smokers may have a limited ability to mount an effective pulmonary immune defense for up to 6 months after smoking cessation.

References


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