Impact of Cigarette Smoke on Clearance and Inflammation after Pseudomonas aeruginosa Infection

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The object of this study was to investigate the impact of cigarette smoke on bacterial clearance and immune inflammatory parameters after infection with Pseudomonas aeruginosa in mice. We observed a delayed rate of bacterial clearance in smoke-exposed compared with sham-exposed mice. This was associated with increased inflammation characterized by greater numbers of neutrophils and mononuclear cells in the bronchoalveolar lavage. After infection, we observed increased levels of proinflammatory cytokines (tumor necrosis factor-α, interleukin-1β, and interleukin-6) and chemokines (monocyte chemoattractant protein-1 [MCP-1] and macrophage inflammatory protein-2 [MIP-2]) as well as myeloperoxidase and proteolytic activity in the lungs of smoke-exposed compared with sham-exposed animals. Delayed clearance was associated with increased morbidity and greater weight loss of smoke-exposed mice. After delivery of inactivated bacteria, we observed a similar inflammatory response, clinical score, and tumor necrosis factor-α expression in smoke- and sham-exposed animals, suggesting that increased inflammation and altered clinical presentation are due to the delayed rate of bacterial clearance. Our findings suggest that cigarette smoke affects respiratory immune-inflammatory responses elicited by bacteria. We postulate that altered respiratory host defense may be implicated in smoking-related diseases such as chronic obstructive pulmonary disease.

Keywords: bacterial infection; chronic obstructive pulmonary disease; mice; tobacco

Pseudomonas aeruginosa is an opportunistic Gram-negative bacillus causing both acute and chronic infections in susceptible individuals (1–3). In addition to being a primary pathogen among individuals with cystic fibrosis and bronchiectasis, P. aeruginosa has also been implicated both in stable chronic obstructive pulmonary disease (COPD) and during exacerbations (4–10).

Generally, P. aeruginosa does not infect healthy tissue (11). It becomes pathogenic only when introduced into areas devoid of normal defense mechanisms or during mixed infections (2, 3). That cigarette smoking predisposes to P. aeruginosa colonization and pulmonary infections (4–10, 12) may suggest that smoking impairs respiratory host defense mechanisms. Indeed, smoking has been shown to suppress antibacterial activities of macrophages (13–15) and delay clearance of microbial agents from the lungs (15–17). Little is known, however, how this altered host defense affects immune inflammatory processes in the lungs after bacterial exposure.

The objective of this study was to investigate the impact of cigarette smoke on bacterial clearance, immune inflammatory processes, and clinical presentation of mice after acute P. aeruginosa infection. We observed a decreased rate of bacterial clearance in cigarette smoke-exposed animals. This was associated with increased inflammation and deteriorated clinical presentation in experimental animals. The changes were due to a combination of bacterial infection and smoke exposure, as tobacco smoke alone neither affected the clinical status nor caused overt lung inflammation under our experimental conditions. Based on our findings, we suggest that a detailed understanding of host/bacteria interactions in the context of cigarette smoke may provide novel insight into the pathogenesis of smoking-related diseases such as COPD. Some of the results of this study have been previously reported as an abstract (18).

METHODS

Animals
Female C57BL/6 mice (6–8 weeks old) were purchased from Charles River Laboratories (Montreal, PQ, Canada). All experiments described in this study were approved by the Animal Research Ethics Board of McMaster University.

Smoke Exposure Protocol
Experimental animals were exposed to two 1R1 or 1R3 reference cigarettes (Tobacco and Health Research Institute, University of Kentucky, Lexington, KY), 5 days per week, over the period of 6–8 weeks using a mainstream cigarette smoke exposure system (19, 20).

P. aeruginosa Preparation and Infection
One × 10^6 cfu of P. aeruginosa (PA103) (provided by Dr. Douglas Storey, University of Calgary, Calgary, AB, Canada) were administered intranasally in a total volume of 35 μl of phosphate-buffered saline (PBS) into isoflurane-anesthetized animals. Bacteria were delivered within 30–60 minutes after cigarette smoke exposure. Details of inactivation of bacterial stock are described in an online supplement. Animals were inoculated with an equivalent of what would have been 1 × 10^6 and 1 × 10^5 cfu in 35 μl of PBS.

Clinical Status Assessment and Body Weight Measurement
The health status of experimental animals was assessed based on appearance, provoked and unprovoked behavior, hydration status, clinical signs, and breathing pattern, as required by the Canadian Council on Animal Care Guidelines on Choosing an Appropriate Endpoint in Experiments Using Animals for Research, Teaching and Testing. Scoring of appearance was based on grooming, piloerection, nasal and ocular discharge, and stature of animals. Behavioral changes were based on activity, and clinical signs were based on breathing pattern. The clinical score was derived by adding the individual scores. Additional detail is provided in an online supplement. To reduce biases, cage cards were turned after infection of animals and assigned a number code that was broken after completion of the sacrifice. Scoring was performed by the experimenter and confirmed on two separate occasions by two individuals that were blinded to the experimental groups. Body weight was determined by weighing animals before and after inoculation with...
bacteria. Data are expressed as the percentage weight change compared with before bacterial delivery.

Collection and Measurement of Specimens
Bronchoalveolar lavage (BAL) and collection of peripheral blood was performed as described in detail previously (21). For bacterial clearance, the left lung lobe was homogenized after BAL. Bacterial load was assessed in lung homogenates, and BAL fluid and counts from lung homogenates and BAL were combined. A detailed description of all of the methods used is provided in an online supplement. For histologic evaluation, lungs were inflated with 10% formalin at a constant pressure of 20 cm H2O and embedded in paraffin. Three-micrometer-thick sections were stained with hematoxylin and eosin.

In Vitro Lipopolysaccharide Stimulation and Phagocytosis
Assay of Alveolar Macrophages
BAL cells were plated at 1 × 10⁵ cells/well in a flat-bottom 96-well plate at 37°C for 2 hours. Nonadherent cells were removed, and adherent alveolar macrophages were incubated in medium alone or 2-μg/ml lipopolysaccharide (LPS) (Escherichia coli O55:B5; Sigma-Aldrich, Oakville, ON, Canada) for 24 hours. For phagocytosis assays, 1.5 ml lipopolysaccharide (LPS) (Escherichia coli O55:B5; Sigma-Aldrich, Oakville, ON, Canada) and 2 ml lipopolysaccharide (LPS) (Escherichia coli O55:B5; Sigma-Aldrich, Oakville, ON, Canada) were added to each well. Cigarette smoke was added to the medium for 24 hours.

Further detail on the method is provided in an online supplement.

Measurements of Cytokines and Chemokines
ELISA kits for murine interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), IL-6, macrophage inflammatory protein-2 (MIP-2), and monocye chemoattractant protein-1 (MCP-1) were purchased from R&D Systems (Minneapolis, MN). Measurements were performed according to the manufacturer’s protocol. The sensitivity of IL-1β was less than 3.0 pg/ml, TNF-α was less than 5.1 pg/ml, IL-6 was less than 3.1 pg/ml, MIP-2 was less than 1.5 pg/ml, and MCP-1 was less than 2.0 pg/ml.

Myeloperoxidase Analysis
Myeloperoxidase activity was determined in lung homogenates using a standard protocol with minor modifications (22). Details of the experimental procedures are described in an online supplement.

Zymography
Casein and gelatin zymography were performed on pooled supernatants from lung homogenates of smoke- and sham-exposed animals before and after infection with P. aeruginosa. Additional detail on methods is provided in the online supplement. Myeloperoxidase activity was determined in lung homogenates using Image Quant Version 5.2 (GE Healthcare, Piscataway, NJ).

Data Analysis
Data are expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance with the Fisher least significant difference (LSD) post hoc test unless otherwise stated. Differences were considered statistically significant when p < 0.05.

RESULTS
Bacterial Clearance
To assess the impact of cigarette smoke exposure on bacterial clearance, mice were exposed to two cigarettes daily, 5 days per week, over a period of 6 to 8 weeks. To control for handling, animals were placed into restrainers only (sham exposure). Cigarette smoke- and sham-exposed animals were intranasally inoculated with 10⁵ cfu of viable P. aeruginosa. Figure 1 shows that before inoculation, no bacteria were detected in sham- and smoke-exposed mice. Although smoke-exposed animals compared with sham-exposed mice had a significantly greater bacterial load in the lungs 4 and 12 hours after bacterial inoculation, both groups of animals cleared the bacteria by 24 hours. The insert in Figure 1 shows that similar numbers of P. aeruginosa were cultured from the lungs of sham- and smoke-exposed animals 30 minutes after inoculation.

Clinical Status and Body Weight of Experimental Animals
Increased bacterial burden observed in smoke-exposed mice after bacterial inoculation was associated with greater morbidity (Figure 2A). Clinical status was assessed as described in detail in the online supplement. Before infection, we did not observe differences in clinical presentation between smoke- and sham-exposed animals. However, cigarette smoke exposure significantly deteriorated the clinical presentation of smoke-exposed compared with sham-exposed animals for up to 48 hours after bacterial infection. At 72 hours, the clinical presentation between the groups was similar to that observed before infection.

To corroborate the differences in clinical status, body weight of experimental animals was measured. Before bacterial inoculation, the weight of sham- and cigarette smoke-exposed animals was comparable (28.2 ± 3.5 g for smoke-exposed and 27.9 ± 3.1 g for sham-exposed animals). After bacterial delivery, smoke-exposed mice exhibited a significantly greater weight loss at 12, 24, and 48 hours compared with sham-exposed animals (Figure 2B).

Cellular Content in the BAL and the Peripheral Blood
To address whether exacerbated clinical status observed in cigarette smoke-exposed animals after infection with P. aeruginosa was associated with increased inflammation, we assessed the cellular content in the BAL (Figure 3). Peak inflammation in the BAL was observed 12 hours after bacterial inoculation in both smoke- and sham-exposed animals. Compared with sham-
Figure 2. Clinical status and body weight loss after *P. aeruginosa* inoculation. Mice were either sham- or cigarette smoke–exposed for 6–8 weeks and inoculated with 1 × 10^4 cfu *P. aeruginosa*. (A) The clinical score was assessed using observation criteria described in detail in METH-ods. Data represent mean ± SEM; 0, n = 30–31; 4 and 12, n = 24–25; 8 and 24, n = 18–19; 48, n = 9–10; 72, n = 4. (B) Body weight loss was determined by weighing animals before and after inoculation with bacteria. Data are expressed as percentage changes in weight compared with before bacterial delivery. Data represent mean ± SEM (n = 5–15). Statistical analysis in A and B was performed using one-way analysis of variance with the Fisher LSD post hoc test. *p < 0.05 smoke compared with sham.

Exposed mice, smoke-exposed animals had significantly greater total cell numbers 12 and 24 hours after *P. aeruginosa* inoculation. Increased inflammation was characterized by a significant increase in neutrophils in smoke-exposed compared with sham-exposed mice 12 hours after bacterial delivery. At 24 hours, we observed significantly more mononuclear cells in smoke-exposed compared with sham-exposed animals.

Histologic assessment of lung tissues revealed no overt inflammation in either sham- or smoke-exposed animals before bacterial infection (Figures 4A and 4B). At 12 hours after delivery of *P. aeruginosa*, we observed an inflammatory infiltrate predominantly around larger airways in sham- and smoke-exposed animals (Figures 4C and 4D). The inflammatory infiltrate was characterized by mononuclear cells and neutrophils in both experimental groups (Figures 4E and 4F). To quantify tissue inflammation, we next assessed myeloperoxidase activity in lung homogenates. Both sham- and smoke-exposed animals showed significantly increased myeloperoxidase activity 12 hours after delivery of bacteria compared with before inoculation (Table 1). Myeloperoxidase activity was significantly greater in smoke-exposed compared with sham-exposed animals at the 12-hour time point corroborating increased inflammation in the BAL (Figure 3).

Assessment of cellular content in the peripheral blood, measured before and after bacterial inoculation, revealed comparable numbers of total cells, mononuclear cells, and neutrophils between sham- and smoke-exposed animals (Table 2). However, in both sham- and smoke-exposed animals, we observed a significant increase in neutrophils 24 hours after bacterial delivery. Peripheral blood neutrophilia resolved by 48 hours in both groups (data not shown).

**Cytokine, Chemokine, and Proteolytic Enzyme Expression in Lung Homogenates**

To investigate whether increased inflammation observed in cigarette smoke-exposed mice was associated with an altered cytokine and chemokine expression in response to bacterial infection, animals were either sham or cigarette smoke exposed for 6 to 8 weeks and inoculated with viable 10^4-cfu *P. aeruginosa*. Proinflammatory cytokines and chemokines were measured in lung homogenates. Figure 5 shows that compared with sham-exposed mice, smoke-exposed animals had significantly greater levels of the proinflammatory cytokines TNF-α, IL-1β, and IL-6, as well as the chemokines MCP-1 and MIP-2, 12 hours after bacterial inoculation. Levels of MCP-1 were also significantly increased at 4 hours.

Given the increased inflammation and expression of proinflammatory mediators, we next performed zymography to investigate the impact of cigarette smoke on proteolytic activity in the lungs. Using casein as a substrate, we observed greater proteolytic activity around 85 and 28 kD in smoke-exposed animals 4, 12, and 24 hours after bacterial inoculation (Figure 6A). Gelatine zymography also revealed greater proteolytic activity around 85 kD, whereas decreased activity was observed around 68 kD in smoke-exposed compared with sham-exposed animals at all time points (Figure 6B). Quantification of bands at the 12-hour time point using densitometric analysis confirmed that proteolytic activity around 85 kD (casein and gelatin zymography) and 28 kD (casein zymography) was significantly increased, whereas activity around 68 kD was decreased in smoke-compared with sham-exposed animals (Table 3).


**In Vitro Cytokine Production and Phagocytosis by Alveolar Macrophages**

To investigate whether elevated levels of proinflammatory cytokines in smoke-exposed animals were due to altered macrophage responsiveness to bacterial agents, alveolar macrophages were isolated after 8 weeks of smoke exposure and stimulated *in vitro* with LPS. Figure 7A shows that alveolar macrophages isolated from smoke-exposed mice compared with sham-exposed animals produced significantly less TNF-α and IL-6 in response to LPS stimulation. Similarly, after infection with *P. aeruginosa*, alveolar macrophages from smoke-exposed compared with sham-exposed animals produced less TNF-α and IL-6 in response to LPS (data not shown).

To examine whether smoke exposure altered macrophage phagocytic activity, animals were sham or cigarette smoke exposed for 8 weeks. Alveolar macrophages were isolated, overlaid with hIgG-opsonized latex beads, and allowed to phagocytose for 1 hour. Figure 7B shows that the percentage of macrophages phagocytosing one or more beads and the average number of beads associated with each macrophage were similar between smoke- and sham-exposed animals.

**INOCULATION WITH INACTIVATED P. aeruginosa**

We next investigated whether increased inflammation observed in smoke-exposed animals depends on inoculation with live, intact *P. aeruginosa*. Figure 8A shows that the total cell number as well as mononuclear cells and neutrophils were similar between smoke- and sham-exposed animals after inoculation with 10⁶ cfu of inactivated bacteria. Moreover, a similar clinical score (Figure 8B) and a weight loss of approximately 0.5 g (data not shown) were observed in smoke- and sham-exposed mice. Levels of TNF-α expression were reduced by approximately 40%; however, this decrease did not reach significance (*p = 0.066*) (Figure 8C). At 10⁴ cfu of inactivated bacteria, the BAL cellular profile in sham- and smoke-exposed animals was similar to that observed in sham and smoke-only exposed mice (data not shown). Finally, similar levels of inflammatory cells and TNF-α were observed between smoke- and sham-exposed animals after inoculation with LPS (data not shown).

**DISCUSSION**

Accumulating data suggest that cigarette smoke compromises the immune system (23, 24). As a consequence, infectious agents may be cleared ineffectively and elicit prolonged inflammation. Such processes may contribute to the pathogenesis of smoking-related diseases.
related diseases such as COPD. In this study, we investigated the impact of cigarette smoke exposure on bacterial clearance and immune-inflammatory processes after infection with *P. aeruginosa* in mice.

To expose mice to cigarette smoke, we used a mainstream cigarette smoke exposure system typically used to study smoke-induced emphysema in rodents. The system was first described for guinea pigs (19) and was later modified to accommodate mice (20). In this study, animals were exposed to cigarette smoke for 6–8 weeks because we previously observed profound differences in antiviral responsiveness at this time point (25). To model bacterial infection, we elected to use *P. aeruginosa* partly because of its clinical relevance to COPD (4–10) and partly because the bacillus typically infects areas with compromised defense mechanisms (1–3).

We observed a lower rate of bacterial clearance in cigarette smoke–exposed animals compared with sham-exposed animals after *P. aeruginosa* infection. This finding is in agreement with previous studies (15–17) and demonstrates that cigarette smoke suppresses respiratory antibacterial host defense. None of these studies, however, assessed the impact of altered bacterial clearance on immune inflammatory parameters and clinical presentation. Delayed clearance was associated with increased inflammation observed in smoke-exposed mice after an infection. Cigarette smoke may predispose the lung to an increased inflammatory burden. Moreover, an altered rate of bacterial clearance and increased inflammation were associated with a significant deterioration of the clinical status of experimental animals. That smoke alone neither affected the clinical status nor caused overt lung inflammation suggests that the observed changes were due to the combined effects of bacterial infection and smoke exposure. Increased bacterial load in the lungs of smoke-exposed animals was not a result of differences in bacteria delivered because equal numbers of *P. aeruginosa* were recovered from both groups 30 minutes after inoculation.

At least two mechanisms may contribute to the elevated inflammation observed in smoke-exposed mice after *P. aeruginosa* infection. Cigarette smoke may predispose the lung to an increased immune responsiveness to bacterial agents. According to this paradigm, we would expect greater inflammation in animals exposed to LPS or inactivated bacteria. Alternatively, increased inflammation may be the consequence of delayed bacterial clearance. In this case, increased inflammation would be the consequence of prolonged and more robust interaction between host and pathogen. That similar responsiveness between smoke-

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**TABLE 2. PERIPHERAL BLOOD CELLULAR PROFILE**

<table>
<thead>
<tr>
<th></th>
<th>Total Cells</th>
<th>Mononuclear Cells</th>
<th>Neutrophils</th>
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<tr>
<td></td>
<td>Cells × 10⁶</td>
<td>Cells × 10⁶</td>
<td>Cells × 10⁶</td>
</tr>
<tr>
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<td>Smoke</td>
<td>Sham</td>
<td>Smoke</td>
</tr>
<tr>
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<td>2.7 ± 0.4</td>
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</tr>
<tr>
<td>12</td>
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<td>3.6 ± 0.6</td>
<td>5.8 ± 1.2</td>
</tr>
<tr>
<td>24</td>
<td>4.6 ± 0.7</td>
<td>3.4 ± 0.6</td>
<td>11.3 ± 2.3*</td>
</tr>
</tbody>
</table>

Mice were either sham- or cigarette smoke–exposed for 6–8 weeks and inoculated with 1 × 10⁴ cfu *P. aeruginosa*. Data show total cell number, mononuclear cells, and neutrophils in the peripheral blood before and after inoculation with *P. aeruginosa* (mean ± SEM; n = 6; 12 hours, n = 10; 24 hours, n = 9). Statistical analysis was performed using one-way analysis of variance with the Fisher LSD post hoc test.

* Represents p < 0.05 sham compared with sham at 0 hours and 12 hours.
† Represents p < 0.05 smoke at 24 hours compared with sham at 0 and 12 hours.

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**Figure 5.** Proinflammatory cytokines and chemokines expression in the lungs after *P. aeruginosa* inoculation. Mice were either sham- or cigarette smoke–exposed for 6–8 weeks and inoculated with 1 × 10⁴ cfu *P. aeruginosa*. Cytokines and chemokines were measured in lung homogenates by commercial ELISA. Data represent mean ± SEM. Tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β: 0 hours, n = 3; 4 hours, n = 6; 12 hours, n = 9–10; 24 hours, n = 8–9. IL-6, MCP-1, and MIP-2: 0 hours, n = 3; 4 hours, n = 6; 12 hours, n = 9; 24 hours, n = 8–9. Statistical analysis was performed using one-way analysis of variance with the Fisher LSD post hoc test; *p < 0.05 smoke compared with sham.
Bands 1 and 2 correspond to caseinolytic activity around 85.9 and 28.4 kD; bands 1–5 represent bands denoted in Figures 6A and 6B. Recognized as an important feature of inflammatory processes and is involved in cell recruitment and repair. Using zymography, we observed greater proteolytic activity at 85 and 28 kD in smoke-compared with sham-exposed animals after bacterial inoculation. These bands likely reflect matrix metalloproteinase-9 (MMP-9) and neutrophil elastase, respectively, based on molecular weight and substrate specificity. We postulate that similar mechanisms may contribute to the elevated proteolytic burden observed in patients with COPD (28). Interestingly, gelatin zymography revealed decreased proteolytic activity around 68 kD in smoke-exposed animals before and after bacterial inoculation. Based on substrate specificity and molecular weight, this band may reflect MMP-2 activity. However, this needs to be confirmed using Western blot analysis. Furthermore, it is unclear whether this decreased proteolytic activity is associated with the phenotype of smoke-exposed animals.

Although both sham- and smoke-exposed animals lost weight after inoculation with P. aeruginosa, we observed a significantly greater and more sustained weight loss in mice exposed to cigarette smoke. Increased weight loss may be a consequence of the temporary decrease in food and water intake or due to a direct effect of increased expression of proinflammatory cytokines such as TNF-α on metabolism. In support of the latter, findings by van Heeckeren and colleagues suggest that expression of proinflammatory mediators play a role in the weight loss associated with P. aeruginosa infection in mice and body composition analysis indicated that weight loss was due in part to a reduction in muscle mass (29). Regardless of the underlying mechanisms, the increased weight loss observed in smoke-exposed animals after bacterial infection corroborates the worsening in clinical score. Alveolar macrophages and polymorphonuclear leukocytes are believed to be the principal components of pulmonary bactericidal system against P. aeruginosa (15, 30–32). Delayed clearance may, therefore, be a consequence of an immunosuppressive

### TABLE 3. DENSITOMETRY ANALYSIS

<table>
<thead>
<tr>
<th></th>
<th>Sham Mean ± SEM</th>
<th>Smoke Mean ± SEM</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>12.3 ± 0.2</td>
<td>19.0 ± 0.9*</td>
</tr>
<tr>
<td>2</td>
<td>1.8 ± 0.2</td>
<td>4.1 ± 0.1*</td>
</tr>
<tr>
<td>3</td>
<td>21.7 ± 0.5</td>
<td>23.7 ± 0.7</td>
</tr>
<tr>
<td>4</td>
<td>56.5 ± 1.7</td>
<td>69.0 ± 2.9*</td>
</tr>
<tr>
<td>5</td>
<td>25.3 ± 0.7</td>
<td>17.7 ± 0.3*</td>
</tr>
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</table>

Mice were either sham- or cigarette smoke–exposed for 6–8 weeks and inoculated with 1 × 10⁶ cfu P. aeruginosa. Casein and gelatin zymographies were run on triplicates of pooled lung homogenates collected from smoke- and sham-exposed animals 12 hours after bacterial administration. Data represent ([mean ± SEM] × 10⁻¹⁰) of volume (intensity) qualified in inverted images using Image Quant Version 5.2. Numbers 1–5 represent bands denoted in Figures 6A and 6B. Bands 1 and 2 correspond to caseinolytic activity around 85.9 and 28.4 kD; bands 3–5 correspond to gelatinolytic activity around 184.5, 85.9, and 68.8 kD. * Represents p < 0.05 smoke compared with sham.
effect of cigarette smoke on macrophage and polymorphonuclear leukocytes function. In our study, macrophages isolated from smoke exposed animals showed significantly decreased production of both TNF-\( \alpha \) and IL-6 in response to LPS. This is in agreement with previous observations (33–35) and further supports the notion that mainstream tobacco smoke suppresses macrophage function (13–15). Decreased cytokine production by macrophages may also explain why we observed reduced levels of TNF-\( \alpha \) expression in smoke exposed animals after delivery of inactivated bacteria.

That we did not observe an impact of cigarette smoke on phagocytic activity of alveolar macrophages suggests that macrophage-independent mechanisms may contribute to delayed clearance. Among them, impaired mucociliary clearance (36, 37) and/or altered expression of components of the surfactant system (38–40) have been suggested. Indeed, cigarette smoking affects mucociliary clearance and leads to impaired tracheobronchial clearance of microorganisms (41). Cigarette smoke also decreases both phospholipid fraction and surfactant-associated proteins A and D in BAL (42, 43). It is noteworthy that mice lacking surfactant-associated protein A have significantly greater pulmonary loads of P. aeruginosa than wild-type mice, which is associated with increased concentrations of TNF-\( \alpha \), IL-6, and MIP-2 (39). On-going studies in our laboratory aim to assess mechanisms that contribute to delayed bacterial clearance.

Based on these findings, we postulate that inflammation and lung injury observed in both smokers who are asymptomatic and patients with COPD may be due in part to ineffective clearance of microbial agents. Prolonged presence of bacteria likely amplifies and prolongs immune–inflammatory responses; the wear-and-tear associated with increased inflammation may ultimately lead to injury and loss of lung function (44, 45). Hence, the pathogenesis of COPD may be in part due to the impact of cigarette smoke on respiratory defense mechanisms. Furthermore, altered responsiveness to microbial agents may also underlie exacerbations observed in more advanced COPD.

That current pharmacologic therapy for other airway inflammatory diseases such as asthma appears to have relatively little therapeutic impact in COPD underscores the need for novel experimental paradigms and therapeutic approaches. Here, we present data that cigarette smoke impairs bacterial clearance leading to increased inflammation and morbidity of experimental animals. Based on our findings, we postulate that a detailed understanding of host/bacteria interactions in the context of cigarette smoke may provide novel insight into the pathogenesis of smoking-related diseases such as COPD.

Conflict of Interest Statement: A.G.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; M.A.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; C.S.R. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; S.I.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; S.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; M.R.S. received $45,000 in 2001 and 2002 from AstraZeneca as research grants to pursue studies on the impact of cigarette smoke on respiratory host defense mechanisms and $35,000 from Millennium in 2002 for generating biological samples from smoke-exposed animals.

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References


![Figure 8. Airway inflammation, clinical status, and TNF-\( \alpha \) expression after inoculation with inactivated P. aeruginosa. Mice were either sham- or cigarette smoke–exposed for 6–8 weeks and inoculated with 1 \times 10^6 cfu inactivated P. aeruginosa. Data show (A) total cell number, mononuclear cells, and neutrophils in the BAL before and 12 hours after inoculation with bacteria, (B) clinical status of sham and smoke groups before and 12 hours after inoculation with bacteria, and (C) levels of TNF-\( \alpha \) expression in lung homogenates (mean ± SEM, n = 6). Statistical analysis was performed using one-way analysis of variance with the Fisher LSD post hoc test, where *p < 0.05.](image-url)


