Cigarette Smoking Increases Neutrophil Formyl Methionyl Leucyl Phenylalanine Receptor Numbers*

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Study objectives: The purpose of this study was to explore the relationship between cigarette smoking and COPD on the number of formyl methionyl leucyl phenylalanine (FMLP) receptors on peripheral neutrophils.

Design and participants: Three groups of subjects were studied: subjects with COPD (n = 13), healthy smokers (n = 6), and healthy nonsmokers (n = 6).

Interventions: Fifty milliliters of venous blood were collected from each subject, and neutrophils were isolated. Neutrophil FMLP receptor numbers were determined by incubating with tritiated FMLP at six doubling concentrations from 1.4 to 45 pmol. Three of the subjects from group 1 (the COPD group) were current smokers, and we elected to analyze these subjects as a separate group.

Measurements and results: The analysis of variance looking at the three factors—FMLP, COPD and smoking—showed significant differences among levels of FMLP (p = 0.0001), as would be expected, and also overall smoking vs nonsmoking (p = 0.0001) and COPD vs non-COPD (p = 0.02). Within each level of FMLP, there was only one instance of a significant difference between COPD nonsmokers and normal nonsmokers, and no instance of a significant difference between COPD smokers and normal smokers. At five of the six concentrations of tritiated FMLP, smoking was a significant factor.

Conclusions: This study suggests that the overriding influence on peripheral neutrophil FMLP receptor numbers is current smoking rather than the presence of COPD.

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Key words: COPD; neutrophils; smoking

Abbreviations: DLco = diffusing capacity of the lung for carbon monoxide; FMLP = formyl methionyl leucyl phenylalanine; PBS = phosphate-buffered saline solution; PMN = polymorphonuclear leukocyte; RV = residual volume

COPD is a major health problem for which a significant smoking history is known to be the predominant risk factor. However, only a small percentage of smokers acquire COPD, and factors that make these smokers acquire COPD and enable its progression are unknown.

One controversial factor proposed to be involved in the etiology and progression of COPD is bacterial infection of the lower respiratory tract. Sethi proposed that impairment to mucociliary clearance caused by smoking enable bacteria to colonize the lower respiratory tract. Bacterial products then produce further damage, enhancing the ability of the bacteria to persist and eventually cause chronic, irreversible damage.

Formyl methionyl leucyl phenylalanine (FMLP) is a tripeptide produced by bacteria as a byproduct of protein synthesis. Receptors for this tripeptide are present on neutrophils and macrophages, the numbers of which are found to be increased in the lavage fluid from cigarette smokers and subjects with chronic bronchitis and emphysema. Activation of the FMLP receptor on these cells leads to chemotaxis, vascular adherence, and induction of the respiratory burst with the release of oxidative products proposed to contribute to the tissue damage seen in...
COPD. Thus, theoretically, a mechanism exists by which bacterial infection of the lower respiratory tract produces increased concentrations of FMLP, which in turn both attracts macrophages and neutrophils to the lungs and stimulates them to release products causing tissue damage.

Cigarette smoking, besides reducing the defenses of the respiratory tract against bacterial infection has also been shown to directly alter such functions of neutrophils as chemotaxis and oxidative metabolism.\(^5^,6\) In vitro, these alterations have been shown to be via changes to the number and affinity of receptors\(^7^,8\) for chemotactic peptides. Thereby, a mechanism exists by which, with the combination of tobacco smoke increasing the number of FMLP receptors on polymorphonuclear leukocytes (PMNs) and bacterial infection increasing the source of FMLP, a significant increase in tissue damage could be expected.

In 1994, Stockley et al\(^9\) reported increased neutrophil FMLP receptor numbers in subjects with emphysema compared to age-matched control subjects that was unrelated to current smoking status. The numbers in this study were relatively small and complicated by the inclusion of smokers, ex-smokers, and nonsmokers in both the control and emphysema groups. The purpose of the study reported here was to further explore the relationship between cigarette smoking and COPD on the number of FMLP receptors on peripheral neutrophils.

**Materials and Methods**

**Subjects**

Three groups of subjects were studied. The first group consisted of 13 patients admitted to hospital with an exacerbation of chronic bronchitis, including current smokers and ex-smokers. The diagnosis was made by a respiratory physician and based on clinical history and lung function test results (Table 1). The lung function test criteria indicating COPD were a subject having two of the following results: a FEV\(_1\)/FVC ratio < 70%, a residual volume (RV) percentage of predicted > 120%, and a diffusing capacity of the lung for carbon monoxide (DLco) percentage of predicted < 70%.

For control subjects, we studied 12 healthy volunteers, of whom 6 subjects were current smokers (group 2) and 6 subjects were nonsmokers (group 3). None had a history of chronic disease or current infection. Smoking history was determined for every subject. Lung function tests were performed on all subjects, and their characteristics are shown in Table 1.

**Isolation of Peripheral Neutrophils**

Fifty milliliters of venous blood were collected from each subject into heparinized tubes. Twenty milliliters of this blood were layered carefully onto 20 mL of Polymorph Prep (Clinical Data; Sydney, NSW, Australia) and spun at 1,850 revolutions per minute for 30 min. The PMNs were harvested, mixed with an equal volume of 0.45% NaCl to restore osmolarity. This was further diluted with phosphate-buffered saline solution (PBS) [without Mg and Ca; 2:1 V] and spun at 1,400 revolutions per minute for 10 min. To the supernatant was added 5 mL of cold lysing buffer (NH\(_4\)Cl 155 mM; KHCO\(_3\), 10 mM; ethylenediamine tetra-acetic acid, 0.14 mM), and the solution was mixed by multiple pipetting. After reaching room temperature, the cells were resuspended at 1,300 revolutions per minute for 5 min. The supernatant was mixed with 1 mL of PBS solution, and a cell count and trypan blue test performed to determine cell viability. Neutrophils were harvested, mixed with 1 mL of PBS, and a cell count and viability test were performed before freezing slowly to \(-30^\circ\)C with cryomix (35 mL of RPMI media, 10 mL of fetal calf serum, and 5 mL of dimethylsulfoxide).

**Determination of Neutrophil FMLP Receptor Numbers**

Following the method of Codd and Bridges,\(^10\) neutrophils were thawed at 37°C in a water bath. Neutrophils were recounted and viability reassessed, and then made up to a concentration of 8 \(\times\) 10\(^6\) cells per milliliter of incubation buffer (KH\(_2\)PO\(_4\), 1.7 mM; Na\(_2\)HPO\(_4\), 8 mM; NaCl, 117 mM; CaCl\(_2\), 0.15 mM; and MgCl\(_2\), 0.5 mM). One hundred milliliters of buffer containing cells were added to 25 L of doubling doses of tritiated FMLP (Amrad Biotech Pharmacia; Richmond, VIC, Australia) from 1.4 to 45 pmol. A control tube contained unlabeled FMLP (Auspep; Melbourne, VIC, Australia). The microcentrifuge tubes were incubated in a shaking water bath at 37°C for 12 min before incubation was terminated by filtration through a glass fiber/cellulose filter with a 4 \(\times\) 5-mL wash with ice-cold incubation buffer. Filters were then dried and counted in a scintillation counter.

**Statistics**

Differences between subject characteristics were tested using analysis of variance and Fisher post hoc. Data for the neutrophil FMLP receptor numbers was logged prior to performing analysis.

**Table 1—Subject Characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group 1, COPD (n = 13)</th>
<th>Group 2, Smokers (n = 6)</th>
<th>Group 3, Nonsmokers (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>75.3 ± 2.0†</td>
<td>60.0 ± 2.2</td>
<td>62.8 ± 1.8</td>
</tr>
<tr>
<td>Female gender, No.</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Pack-years</td>
<td>54.2 ± 7.8</td>
<td>34.4 ± 4.5</td>
<td>0†</td>
</tr>
<tr>
<td>FEV(_1)/FVC</td>
<td>0.43 ± 0.06†</td>
<td>0.69 ± 0.02</td>
<td>0.76 ± 0.05</td>
</tr>
<tr>
<td>DLco, % predicted</td>
<td>36.7 ± 2.9†</td>
<td>74.4 ± 3.2</td>
<td>74.0 ± 9.0</td>
</tr>
<tr>
<td>RV, % predicted</td>
<td>179.2 ± 17.1†</td>
<td>108.8 ± 10.8</td>
<td>76.5 ± 6.5</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD unless otherwise indicated. †p ≤ 0.05.
Results

Subjects

There were significant differences in the mean age between the patients admitted with COPD and both control groups (Table 1). There were also, by definition, significant differences in pack-years between the nonsmokers and smokers both with and without COPD. The lung function tests revealed a significant difference in the FEV₁/FVC percentage of predicted between the patients with COPD and both control groups and likewise for RV percentage of predicted and DLCO percentage of predicted, as expected.

Neutrophil FMLP Receptor Numbers

Individual Lineweaver-Burk plots showing the ratio of amount of radioactive FMLP bound vs the concentration used gave an inverse regression line \((r > 0.9)\) for all groups, suggesting a single class of receptor. Three of the subjects from group 1 (the COPD group) were current smokers, and we elected to analyze these subjects as a separate group. The reported period for not smoking for the COPD ex-smokers was 24.3 ± 4.0 years. Receptor numbers for the four groups at the six concentrations of FMLP are shown in Figure 1. The analysis of variance looking at the three factors—FMLP, COPD, and smoking—showed significant differences among levels of FMLP \((p = 0.0001)\), as would be expected, and also overall smoking vs nonsmoking \((p = 0.001)\) and COPD vs non-COPD \((p = 0.02)\). The pair-wise comparisons within each level of FMLP showed significant differences at five of the six levels of FMLP (Table 2).

Discussion

This study has examined the effect of smoking and COPD on peripheral neutrophil FMLP receptor numbers. The results clearly show a significant effect of smoking, with the nonsmoking control group having the lowest binding rates and the COPD

![Figure 1. Numbers of bound neutrophil FMLP receptors.](http://publications.chestnet.org/pdfeaccess.ashx?url=/data/journals/chest/21993/ on 05/29/2017)
been shown to alter their activity state. However,

preferential cell activation.

microscopy, and it is hard to explain the results by

observations and why they differ from previous

address the underlying mechanisms to these obser-

highly significant effect. Although this study does not

this was so; however, our results overall suggested no

patients with COPD to have an increased number of

neutrophils. From previous studies, we also expected

increasing increases the number of FMLP receptors on

lung neutrophils, but it is most likely that the

changes we observed in the peripheral neutrophils of

that smokers with greater numbers of FMLP recep-

current smoking rather than the presence of COPD.

It is possible that this does not reflect the state of

lung neutrophils, but it is most likely that the

changes we observed in the peripheral neutrophils of

the smokers would be observed in their neutrophils

present in the lungs.

Clearly, the results reported here show that smoking

increases the number of FMLP receptors on neutrophils. From previous studies, we also expected patients with COPD to have an increased number of FMLP receptors. There was limited evidence that this was so; however, our results overall suggested no highly significant effect. Although this study does not address the underlying mechanisms to these observations, it is of interest to hypothesize about our observations and why they differ from previous studies.

A possible explanation for the increase in neutrophil FMLP receptor numbers in smokers is that these subjects have a chronically colonized lower respiratory tract. There is some evidence for this from studies using a bronchoscopic protected specimen brush technique. This colonization could induce an increase in neutrophil FMLP receptors, with the subsequent release of oxidative products producing tissue damage. Supporting evidence for this is seen in the group 2 smokers that have a slightly reduced FEV1/FVC ratio with probable small airway disease as indicated by the elevated RV percentage of predicted.

An alternative explanation is that smoking itself, by stimulating neutrophils, is able to increase expression of FMLP receptors independent of infection. Stockley et al reported increased neutrophil FMLP receptor numbers in subjects with emphysema compared to age-matched control subjects. In contrast to us, they found this increase was not related to current smoking status. They did, however, find an additive effect of smoking and emphysema. Although our subjects with COPD were not accurately age matched, the ages between the smokers and non-smokers were not significantly different. The numbers in the study by Stockley et al were relatively small and complicated by the inclusion of smokers, ex-smokers, and nonsmokers in both the control and emphysema groups; however, another possible reason for the observed differences between the studies was the clinical state of the subjects. Our group 1 patients with COPD were studied while admitted to hospital with an acute exacerbation. Their lower respiratory tract bacterial load was probably greatly increased, and this may have induced receptor down-regulation. The patients in the study by Stockley et al were observed while in a stable clinical state.

This study suggests that the overriding influence on peripheral neutrophil FMLP receptor numbers is current smoking rather than the presence of COPD. However, to more accurately address the possibility that smokers with greater numbers of FMLP receptors on their neutrophils are more susceptible to the onset of COPD and to greater declines in lung function once afflicted would require a large prospective study.

Table 2—Pair-Wise Comparisons Within Each Level of FMLP

<table>
<thead>
<tr>
<th>Pair-Wise Comparisons Within Each Level of FMLP</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMLP, Doubling Concentrations</td>
<td></td>
</tr>
<tr>
<td>1 COPD smokers vs control nonsmokers</td>
<td>0.02</td>
</tr>
<tr>
<td>Control smokers vs control nonsmokers</td>
<td>0.0001</td>
</tr>
<tr>
<td>COPD nonsmokers vs control nonsmokers</td>
<td>0.02</td>
</tr>
<tr>
<td>2 COPD smokers vs control nonsmokers</td>
<td>0.007</td>
</tr>
<tr>
<td>Control smokers vs control nonsmokers</td>
<td>0.02</td>
</tr>
<tr>
<td>COPD smokers vs COPD nonsmokers</td>
<td>0.02</td>
</tr>
<tr>
<td>Control smokers vs COPD nonsmokers</td>
<td>0.04</td>
</tr>
<tr>
<td>3 COPD smokers vs control nonsmokers</td>
<td>0.02</td>
</tr>
<tr>
<td>Control smokers vs control nonsmokers</td>
<td>0.04</td>
</tr>
<tr>
<td>4 COPD smokers vs control nonsmokers</td>
<td>0.003</td>
</tr>
<tr>
<td>COPD smokers vs COPD nonsmokers</td>
<td>0.02</td>
</tr>
<tr>
<td>Control smokers vs control nonsmokers</td>
<td>0.03</td>
</tr>
<tr>
<td>5 No significant differences</td>
<td></td>
</tr>
<tr>
<td>6 COPD smokers vs control nonsmokers</td>
<td>0.03</td>
</tr>
</tbody>
</table>

References
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1646
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