No Increase in Plasma Histamine During PAF-induced Airway Obstruction in Allergic Asthmatics*

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To investigate the possible role of mast cell or basophil histamine release in mediating platelet-activating factor (PAF) airway obstruction, we studied the effect of inhaled PAF (30 μg, single dose) on plasma histamine, bronchial caliber, and leukocyte and platelet counts in six patients with mild or moderate allergic asthma (mean age, 27±1.3 years; mean FEV₁, 95±5 percent of predicted; mean PC20 methacholine, 1.46±0.36 mg/ml). Specific conductance (SGaw) FEV₁, FEF25-75 percent, differential leukocyte and platelet counts, and plasma histamine (radioimmunoassay) were measured before and 5, 10, 15, and 20 min after PAF inhalation. Mean basal plasma histamine level was 0.25±0.04 ng/ml. Inhalation of PAF caused a fall in SGaw peaking at 5 min (43±9 percent) and a fall in FEV₁ and FEF25-75 peaking at 10 min (19±10 percent and 30±13 percent, respectively). There was also a rapid and transient fall in circulating neutrophils at 5 min (from 3,006±204/mm³ to 2,551±158/mm³, p<0.05) followed by a rebound neutrophilia. In contrast, plasma histamine level did not change significantly at any time measured. Conversely in the same asthmatics, a rapid rise in plasma histamine level (from 0.29±0.03 ng/ml at baseline to 0.53±0.06 ng/ml at 5 min; p<0.01) was observed after an allergenic challenge (Dermatophagoides pteronyssinus) causing a fall in FEV₁, peaking at 10 min (22±4 percent). Thus, inhaled PAF may induce airway obstruction and neutropenia in asthmatics without any significant change of plasma histamine level. These results indicate that it is unlikely that lung mast cells or basophils degranulate during PAF-induced bronchoconstriction.

Materials and Methods

Subjects

Six patients with mild or moderate stable allergic asthma voluntarily for this study. Their age, sex, basal pulmonary function, bronchial responsiveness to methacholine (methacholine provocative concentration causing a 20 percent fall in FEV₁), possible therapy are given in Table 1. Allergic status was determined from both the clinical history and skin prick tests for common aeroallergens: Dermatophagoides pteronyssinus, grass pollen, tree pollen, weed pollen, molds, cat and dog dander, and feathers.

Study Design

The selected volunteers attended the lung function laboratory on four occasions. Bronchial challenges first with methacholine, then with PAF, and finally with allergen were performed in this chronological order at intervals of at least 1 and maximally 4 weeks. Care was taken to make sure that all subjects were free of upper respiratory tract infections for at least 4 weeks before each study period. Treatment with β₂-agonists was stopped at least 12 h before the tests. Caffeine-containing beverages were also stopped during the challenge days. The study was approved by the local ethics committee, and all subjects gave their written informed consent.

Methacholine Challenge

Methacholine chloride solutions (biochemicals) were dissolved in saline solution, stored at 4°C, and used within 14 days after preparation. Bronchial responsiveness was assessed according to the method described by Cockroft et al., with methacholine instead of histamine. Increasing through doubling concentrations ranging from 0.03 mg/ml to 4 mg/ml were used. After baseline FEV₁.
measurement, subjects first inhaled, as a control, aerosolized saline solution through quiet tidal breathing for 2 min. FEV₁, was measured 3 min after the saline solution provocation and was considered as the control value. Provided FEV₁, did not fall by more than 5 percent of the baseline value, a methacholine concentration-response study was carried out. Starting at a concentration of 0.03 mg/ml and doubling the concentration every 5 min until a 20 percent fall in FEV₁, had occurred, subjects inhaled a methacholine solution through quiet tidal breathing for 2 min. The aerosols were delivered by a nebulizer (Hudson) containing 3 ml of solution and driven by compressed air at 8 ml/min. Under these conditions, the nebulizer had an output of 0.3 ml/min and generated an aerosol with particles of 2-μm mass median diameter. FEV₁, was measured by spirometry (Sensor Medic) 3 min after each methacholine concentration. The provocation dose that produced a 20 percent fall in FEV₁, was read off the log dose-response curve by linear interpolation.

**PAF Challenge**

A stock solution and dilutions of PAF were prepared on the morning of each study day. The PAF (1-O-alkyl-2-O-acetyl-sn-glyceryl-3-phosphorylcholine, Sigma) was purchased as a 2 mg/ml solution in chloroform. Chloroform was evaporated and PAF was dissolved in a saline solution containing 2 percent ethanol to give a final concentration of 50 mg/L. A saline solution served as the control. Aerosols were delivered as described above by a nebulizer (Hudson) loaded with 3 ml of PAF solution. Subjects inhaled the prepared solution through quiet tidal breathing for 2 min so that 30 μg of PAF was delivered by the nebulizer. Measurement of specific conductance (SGaw) (plethysmography; Bodytest-Jaeger) followed by those of FEV₁, and expiratory flow between 25 and 75 percent of vital capacity (FEF25-75) (pneumotachography; Pulmonary Calculator System 47804A Hewlett-Packard) were made before and 5 min after saline solution and then at 5, 10, 15, and 20 min after PAF inhalation. The control values were those observed after the inhalation of the saline solution. The variations in SGaw, FEV₁, and FEF25-75 were expressed as a percentage of the control values.

**Allergen Challenge**

After verifying that the original allergen solution gave positive skin prick test (wheal >5 mm at 15 min), bronchial challenge was performed using solutions of *D. pteronyssinus* (Bencard) diluted in saline solution at concentrations ranging from 1/100,000 to 1/10. A concentration-response study was performed for each subject to determine the dose producing a fall in FEV₁, of 15 to 25 percent. Spirometric measurements of FEV₁, (Sensor Medic) were performed 5 min and 15 min after each allergen concentration. Starting at 1/100,000, tenfold increasing concentrations were successively inhaled for 2 min by tidal breathing with a nebulizer (Hudson) until a fall in FEV₁, of at least 15 percent was achieved or a concentration of 1/10 was reached. One week later, a bronchial challenge was performed by giving directly the allergen dose producing a fall in FEV₁, of at least 15 percent. FEV₁, measures were performed before and 5, 10, 15, and 20 min after inhalation of the allergen. The variations in FEV₁, were expressed as a percentage of control value which was that measured after saline solution.

**Blood Sampling and Histamine Assay**

Before starting the inhalation, a catheter was placed in the antecubital vein for sampling blood throughout the procedure. Venous blood was taken into a specimen tube (Vacutainer) containing edetic acid before and 5, 10, 15, and 20 min after PAF or allergen inhalation. Differential leukocyte cells and platelet counts were made with an automatic hematocytometer (Technicon H1) the day of PAF challenge. For histamine determination, venous blood was immediately centrifuged at 4°C and the upper plasma layer was carefully removed and stored at -20°C until analysis. Histamine was measured in triplicate by a specific radioimmunoassay (Immuno-Notech, Marseille, France). In our hands, the intra-assay and interassay coefficients of variation were 6 percent and 8 percent, respectively, and the lower limit detection 0.1 ng/ml.

**Data Analysis**

Results were reported as a mean ± SEM. A paired Student’s t test was used to determine whether the change in one variable during the test time was significant compared with the control value. The correlation between two variables was assessed by simple linear regression. Only p value <0.05 was considered significant.

**RESULTS**

Only one of the six asthmatics studied had mild basal airway obstruction as shown by FEV₁, and FEV₁/FVC of 77 percent and 79 percent of the predicted values, respectively (Table 1). Arithmetic mean PC20M was 1.46 ± 0.36 mg/ml (range 0.05 to 2.6 mg/ml). After PAF inhalation, all subjects experienced cough and throat irritation and two of them had a transient facial flushing. The inhalation of 30 μg PAF induced a rapid fall in SGaw, FEF25-75, and FEV₁, reaching maximal at 10 min, while the fall in SGaw was maximal at 5 min. These three parameters remained reduced during the 20 min after PAF inhalation (Fig 1).

Table 1—Patient Characteristics

<table>
<thead>
<tr>
<th>Subjects/ Age, yr/ Sex</th>
<th>FEV₁, L</th>
<th>% pred</th>
<th>FEV₁/FVC</th>
<th>FEV₁25-75, L</th>
<th>SGaw, L</th>
<th>PC20M, mg/ml</th>
<th>Medications*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/25/M</td>
<td>3.81</td>
<td>77</td>
<td>63</td>
<td>2.69</td>
<td>0.90</td>
<td>0.84</td>
<td>Salbutamol O</td>
</tr>
<tr>
<td>2/26/M</td>
<td>3.98</td>
<td>97</td>
<td>74</td>
<td>2.92</td>
<td>2.06</td>
<td>2.6</td>
<td>Salbutamol R</td>
</tr>
<tr>
<td>3/35/M</td>
<td>3.19</td>
<td>96</td>
<td>87</td>
<td>3.74</td>
<td>0.70</td>
<td>0.05</td>
<td>Cromoglicate R</td>
</tr>
<tr>
<td>4/25/M</td>
<td>5.56</td>
<td>111</td>
<td>80</td>
<td>5.17</td>
<td>0.47</td>
<td>1.8</td>
<td>Salbutamol R</td>
</tr>
<tr>
<td>5/53/M</td>
<td>4.52</td>
<td>86</td>
<td>84</td>
<td>4.89</td>
<td>0.58</td>
<td>1.52</td>
<td>Beclomethasone R</td>
</tr>
<tr>
<td>6/28/M</td>
<td>4.64</td>
<td>102</td>
<td>77</td>
<td>4.41</td>
<td>1.37</td>
<td>2</td>
<td>Salbutamol O</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>4.283 ± 0.333</td>
<td>94.8 ± 4.8</td>
<td>77.5 ± 3.47</td>
<td>96.8 ± 4.5</td>
<td>3.97 ± 0.419</td>
<td>1.02 ± 0.249</td>
<td>1.46 ± 0.36</td>
</tr>
</tbody>
</table>

*O = occasional; R = regular. Other abbreviations in text.
As shown in Table 2, PAF inhalation produced a rapid fall in circulating neutrophils at 5 min (p<0.05) followed by a significant rebound neutrophilia at 10, 15, and 20 min (p<0.01). For all subjects, there was no relationship between the maximal fall in any of the three pulmonary function parameters and the fall in circulating neutrophils.

The mean control plasma histamine level of our six asthmatics was 0.28±0.04 ng/ml (range, 0.14 to 0.40 ng/ml) on the day of PAF challenge. There was no significant change in mean plasma histamine level during bronchospasm induced by 30 μg of inhaled PAF (Fig 2). When considering individual results, even the two subjects who experienced facial flushing did not display an increase in plasma histamine level. The mean control plasma histamine level of the same asthmatics was 0.29±0.03 ng/ml (range, 0.24 to 0.44 ng/ml) on the day of allergen challenge. The inhalation of an appropriate dose of allergen (D. pteronyssinus) caused acute bronchospasm assessed by a fall in FEV1, reaching maximally 22±4 percent at 10 min (Fig 2). After allergen inhalation, there was a rapid rise in plasma histamine level up to 0.53±0.06 ng/ml (range, 0.32 to 0.71 ng/ml) at 5 min (p<0.01) followed by a decrease to 0.35±0.04 ng/ml at 20 min, a level which, however, remained significantly above control (p<0.05).

**DISCUSSION**

This study confirms that inhaled PAF can cause airway obstruction in allergic asthmatics. This airway obstruction is rapid in onset and is localized primarily in larger airways as suggested by the prominent change in SGaw as compared with FEF25-75. This phenomenon has been reported previously by Rubin et al\(^{12}\) both in normal subjects and in asthmatics. Our study also shows that inhalation of PAF produces a rapid and transient fall in circulating neutrophils, while the count

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**Table 2—Effect of Inhaled PAF on Circulating Leukocyte and Platelet Counts**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>3,096±204</td>
<td>2,551±158†</td>
<td>3,136±279‡</td>
<td>3,265±306‡</td>
<td>3,228±331‡</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>2,233±163</td>
<td>2,141±132</td>
<td>2,101±114</td>
<td>2,161±154</td>
<td>2,065±157</td>
</tr>
<tr>
<td>Monocytes</td>
<td>426±50</td>
<td>371±43</td>
<td>418±21</td>
<td>466±28</td>
<td>438±25</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>233±47</td>
<td>235±57</td>
<td>230±49</td>
<td>228±59</td>
<td>240±64</td>
</tr>
<tr>
<td>Basophils</td>
<td>51±25</td>
<td>46±17</td>
<td>40±28</td>
<td>54±26</td>
<td>47±14</td>
</tr>
<tr>
<td>Platelets</td>
<td>219±21</td>
<td>214±22</td>
<td>217±22</td>
<td>216±23</td>
<td>220±22</td>
</tr>
</tbody>
</table>

*Mean ± SEM; cells/mm\(^3\); 10\(^6\) cells/mm\(^3\) for platelets.
†p<0.05; a significant difference compared with baseline.
‡p<0.05; a significant difference compared with 5 min after PAF inhalation.
of other leukocytes and platelets remains stable. A transient neutropenia was also reported in previous studies\textsuperscript{3,13,14} and is thought to be related to the chemotactic activity of PAF on human granulocytes \textit{in vitro},\textsuperscript{15} \textit{ex vivo},\textsuperscript{16} and \textit{in vivo}.	extsuperscript{13} In agreement with Wardlaw et al,\textsuperscript{16} we did not find any relationship between the magnitude of airway obstruction and the neutropenia, which suggests that the two events are independent. The mechanism of PAF-induced airway obstruction might be indirect and remains poorly understood. Indeed, human bronchi \textit{in vitro} only respond to PAF in the presence of platelets.\textsuperscript{4} In other respects, Smith et al\textsuperscript{5} reported a protective effect of chlorpheniramine, an anti-H\textsubscript{1}, against PAF-induced bronchial obstruction and facial flushing, suggesting a role for histamine in this model. Furthermore, in some conditions, PAF was found to be able to release histamine from human basophils.\textsuperscript{6,7,17} particularly in asthmatics.\textsuperscript{18} An increase in plasma histamine level, thought to reflect the activation of lung mast cells or basophils, has been demonstrated previously during bronchospasm caused by an allergen or adenosine.\textsuperscript{8} In our study, we demonstrate, using a specific and sensitive radioimmunoassay,\textsuperscript{11,18} that the same asthmatics developed airway obstruction of quite similar magnitude, as assessed by the fall in FEV\textsubscript{1}, with a very different profile in plasma histamine values. Indeed, whereas allergen-induced bronchospasm was accompanied by a rapid and significant increase in plasma histamine level peaking at 5 min, a slight and nonsignificant decrease in plasma histamine level was observed during PAF-induced airway obstruction. The lack of increase in plasma histamine level was even observed in the two subjects who displayed facial flushing. Our results, in association with the reported inability of ketotifen,\textsuperscript{20} azelastine,\textsuperscript{21} and terfenadine\textsuperscript{14} to prevent PAF-induced bronchospasm, cast doubt on the relevance of histamine in mediating the acute airway obstruction generated by PAF. In this view, the first result reported with chlorpheniramine might be regarded as reflecting a pharmacologic property of this molecule in addition to its classical anti-H\textsubscript{1} activity (perhaps anti-PAF). Because PAF is a potent leukotriene secretagogue in \textit{vitro},\textsuperscript{17,21,22} and because a cysteinyl-leukotriene antagonist was shown to attenuate PAF-induced airway obstruction,\textsuperscript{23} the leukotrienes could be proposed as second mediators playing a role in bronchial responses to PAF. More recently, it has been shown that inhalation of PAF was accompanied by an increase in plasma LTB\textsubscript{4}, emphasizing the functional relationship between these two mediators.\textsuperscript{24}

We conclude that PAF can induce acute bronchospasm and transient neutropenia in allergic asthmatics. Unlike that caused by an allergen, PAF-induced bronchospasm is not associated with an increase in plasma histamine level, making it unlikely that lung mast cells or basophils degenerate in this experimental model.

REFERENCES

20. Lay C, Ollier S, Lav C, Holgate S. Effect of azelastine and ketotifen on the bronchial and skin responses to platelet activat-
No Increase in Plasma Histamine During PAF-induced Airway Obstruction (Louis et al)

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19 Voerkel NF, Worthen S, Reeves JT, Henson PH, Murphy RC. Nonimmunological production of leukotrienes induced by PAF. Science 1982; 218:286-88

