Relation between the bronchial obstructive response to inhaled lipopolysaccharide and bronchial responsiveness to histamine

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Abstract

Background Bronchoconstriction has developed after inhalation of lipopolysaccharide in a dose of 20 μg in asthmatic patients and of 200 μg in normal subjects. This study set out to determine whether the bronchial response to lipopolysaccharide was related to non-specific bronchial responsiveness and atopy.

Methods Sixteen subjects with a fall in specific airway conductance of 40% (PD50 sGaw) after inhaling up to 900 μg histamine inhaled 20 μg lipopolysaccharide (from Escherichia coli type 026:B6) a week after bronchial challenge with a control solution of saline. The bronchial response over five hours was measured as change in FEV1 and area under the FEV1-time curve.

Results FEV1 fell significantly more after lipopolysaccharide than after diluent inhalation, the difference in mean (SE) FEV1, being 4.6% (5.4%); response was maximal 60 minutes after lipopolysaccharide inhalation and lasted more than five hours. Histamine PD20 sGaw and PD50 sGaw correlated with the fall in FEV1 after lipopolysaccharide inhalation. There was no difference in the proportions of responders and non-responders to lipopolysaccharide who were atopic.

Conclusion Lipopolysaccharide induced bronchial obstruction is associated with non-specific responsiveness but not with atopy.

Endotoxins are partly formed from the outer cell membrane of Gram negative bacteria and may be shed into the environment surrounding the bacterium. They are potent proinflammatory substances and when inhaled are able to activate various cells in the respiratory tract, including polymorphonuclear leucocytes, macrophages, and mast cells; they also activate several proinflammatory mediators, including complement, arachidonic acid, and neutrophil enzymes. Lipopolysaccharides (the major part of endotoxin) have been found in commercial and natural house dust extracts, raising the possibility that they may cause problems in asthmatic patients.

In normal subjects inhalation of 200 μg lipopolysaccharide causes bronchoconstriction. A dose of 20 μg causes no response in normal subjects but causes bronchoconstriction in some asthmatic patients, suggesting that non-specific bronchial responsiveness might be one factor determining the bronchial response to lipopolysaccharide.

The aim of the present study was to determine how the response to inhaled lipopolysaccharide (20 μg) is related to bronchial responsiveness to histamine and atopic state.

Methods

SUBJECTS

We selected for the study 16 student volunteers or patients (nine male, seven female; mean age 42.2 (SD 12.6) years) with a PD20 sGaw value (a provocation dose of histamine causing a 40% fall in specific airways conductance) of less than 900 μg. Asthma was defined according to the American Thoracic Society criteria. Twelve patients had mild asthma treated with β2 agonists only; four had perennial rhinitis. The patients’ characteristics are shown in table 1.

No patient had had an acute respiratory infection in the month before the study. Four subjects were smokers and were asked to stop smoking 12 hours before each challenge test. None was receiving treatment with anti-histamine, corticosteroid, methylxanthine, sodium cromoglycate, or non-steroid anti-inflammatory drugs. The asthmatic patients were asked to stop β2 agonist drugs for 12 hours before each test. All subjects had baseline measurements of forced expiratory volume in one second (FEV1) and airway resistance (Raw) within the normal range (that is, the predicted value ± 1.65 SD, as recommended by the Communauté Européenne du Charbon et de l’Acier (CECA)).

Patients were defined as atopic or non-atopic on the basis of a family history (first degree) of allergic disorders, raised total serum IgE (PRIST, Phadebas; normal values <200 IU/ml), raised specific serum IgE (radioimmunoallergosorbent test, Pharmacia), and immediate prick skin test responses to common allergens (Bencard UK).

The study was approved by the ethical committee of Saint-Pierre University Hospital (Université Libre de Bruxelles). Oral informed consent was obtained from each subject.

BRONCHIAL CHALLENGE TESTS

Non-specific bronchial responsiveness was assessed by giving histamine diphasate solu-
Relation between the bronchial obstructive response to inhaled lipopolysaccharide and bronchial responsiveness to histamine

Table 1  Characteristics of the subjects

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Smoke</th>
<th>Atopic</th>
<th>Diagnosis</th>
<th>FEV₁ (% pred)</th>
<th>PD₂₀FEV₁ (µg histamine)</th>
<th>PD₄₀sGaw (µg histamine)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>40</td>
<td>+</td>
<td>+</td>
<td>Asthma</td>
<td>86</td>
<td>46-5</td>
<td>24</td>
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<tr>
<td>2</td>
<td>F</td>
<td>45</td>
<td>+</td>
<td>+</td>
<td>Asthma</td>
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<td>123</td>
<td>40</td>
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<td>F</td>
<td>38</td>
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<td>+</td>
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<td>82</td>
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<tr>
<td>4</td>
<td>M</td>
<td>52</td>
<td>-</td>
<td>-</td>
<td>Asthma</td>
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<td>900</td>
<td>465</td>
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<tr>
<td>5</td>
<td>M</td>
<td>63</td>
<td>-</td>
<td>-</td>
<td>Asthma</td>
<td>72</td>
<td>78</td>
<td>36</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>45</td>
<td>-</td>
<td>+</td>
<td>Asthma</td>
<td>115</td>
<td>529</td>
<td>248</td>
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<tr>
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<td>M</td>
<td>65</td>
<td>-</td>
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<td>900</td>
<td>168</td>
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<td>F</td>
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<td>+</td>
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<td>93</td>
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<tr>
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<td>46</td>
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<td>-</td>
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<td>114</td>
<td>90</td>
<td>66</td>
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<tr>
<td>12</td>
<td>F</td>
<td>26</td>
<td>-</td>
<td>-</td>
<td>Rhinitis</td>
<td>91</td>
<td>900</td>
<td>772</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>31</td>
<td>-</td>
<td>+</td>
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<td>900</td>
<td>360</td>
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<tr>
<td>14</td>
<td>M</td>
<td>32</td>
<td>-</td>
<td>-</td>
<td>Rhinitis</td>
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<td>900</td>
<td>834</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>32</td>
<td>-</td>
<td>-</td>
<td>Rhinitis</td>
<td>106</td>
<td>529</td>
<td>228</td>
</tr>
</tbody>
</table>

*Basal FEV₁, measured on the day the histamine challenge test was carried out.

FEV₁—forced expiratory volume in one second; PD₂₀FEV₁—provocation dose of histamine causing a 20% fall in FEV₁; PD₄₀sGaw—provocation dose of histamine causing a 40% fall in specific airways conductance.

HISTAMINE CHALLENGE

Five puffs of control solution were followed by increasing concentrations (1, 2, 4, 8, 16, 32 µg/ml) of histamine solution. Each dose was given at three minute intervals; FEV₁ and sGaw were measured two minutes after each inhalation and expressed as percentages of the value after the control solution.

The cumulative provocative doses of histamine inducing a 20% fall in FEV₁ (PD₂₀FEV₁) or a 40% fall in sGaw (PD₄₀sGaw) were calculated from the dose-response curve. Histamine challenge tests were performed in the two weeks before the bronchial challenge with lipopolysaccharide solvent.

LIPOPOLYSACCHARIDE CHALLENGE

Subjects were challenged with saline on day 1 and with lipopolysaccharide on day 8, a single blind procedure being used. The test was started at 0830 h. FEV₁ (best of three values) was measured three times at 15 minute intervals and the mean of these values was taken as the baseline value. After inhalation of five puffs of 4 µl each of either solvent or lipopolysaccharide solution (total inhaled lipopolysaccharide dose 20 µg), FEV₁ was measured at 15, 30, 60, 120, 180, 240, and 300 minutes and expressed as a percentage of the baseline value. Patients having a 10% decrease or more in FEV₁ from baseline were admitted overnight for observation; other subjects were discharged home with a telephone contact number in case of deterioration.

Salbutamol 200 µg was given at the end of the histamine and lipopolysaccharide challenges.

ANALYSIS

Results are expressed as means with standard deviations in parenthesis. Differences between groups were tested by Mann-Whitney U test or by χ² test with Yates's correction.

The differences between values of FEV₁ after inhalation of control and lipopolysaccharide solutions were analysed at each time point for each patient. The bronchial response to lipopolysaccharide (from the 15th to the 300th minute) was expressed as the area between the lipopolysaccharide and solvent FEV₁-time response (area LPS 15–300) measured by planimetry (60 minutes = 10% FEV₁). It was also measured as the mean decrease in FEV₁ at each time point (LPS 15–300). A two way analysis of variance (with subject and times as factors) was performed on the difference in FEV₁. When the result of the F test was significant, the difference in FEV₁ was compared with the baseline difference in FEV₁, by the modified t test with the Bonferroni correction.

The significance of the lipopolysaccharide response within subjects was tested by a two way analysis of variance on the FEV₁ changes (factors: times and treatment—that is, lipopolysaccharide or solvent). A patient was defined as a significant responder (R) or a non-responder (NR) according to whether the result of the F test was significant.
Figure 1 Mean (SE) results of the lipopolysaccharide (LPS) bronchial challenge test (■—■) compared with control results (□—□) in the 16 patients studied (F^2_14 = 3.139, p < 0.005; SE indicated by bars).

The relation between log PD_{25}FEV, and log PD_{25}sGaw for histamine and the area LPS 15–300 and LPS 15–300 was assessed by the linear correlation coefficient. A p value below 0.05 was considered significant.

Results

The mean difference in change in FEV, from baseline between solvent and lipopolysaccharide was 4.6% (5.4%) in the 16 patients. The difference was significant at 60 minutes (p < 0.001) and lasted more than five hours (fig 1).

There was a significant inverse relation between the response to lipopolysaccharide and the response to histamine (r = -0.61 and -0.73 for LPS 15–300 v log PD_{25}FEV, (p < 0.02) and log PD_{25}sGaw (p < 0.001); r = -0.64 and -0.74 for area LPS 15–300 v log PD_{25}FEV, (p < 0.01) and log PD_{25}sGaw (p < 0.001).

The group who responded to lipopolysaccharide (n = 8) had a mean fall in FEV, (LPS 15–300 of 8.6% (3.1%) and a maximal fall at 60 minutes of 12.9% (6.8%) (fig 2). Responders and non-responders did not differ significantly in terms of age, sex ratio, baseline FEV, or proportions of smokers and of atopic individuals (table 2). Responders to lipopolysaccharide had lower values than non-responders for PD_{25}FEV, (257 (217) v 723 (305) μg histamine; p = 0.025) and PD_{25}sGaw (81 (71) v 369 (302) μg histamine; p = 0.027).

Discussion

Because of the wide variation in structure and biological activity of endotoxin we studied lipopolysaccharide from E coli as this has shown lung toxicity in vitro and after inhalation in animals. In the present work and man, the response to lipopolysaccharide was greater in non-responders. This is consistent with studies of van der Zwan et al, who showed that inhalation of Haemophilus influenzae endotoxin induced decrease in FEV, only in patients with histamine hyperresponsiveness. In the study of Cavagna et al inhaling 40 μg lipopolysaccharide induced bronchoconstriction only in patients with chronic bronchitis and not in normal subjects. They found that normal subjects may respond to higher doses of lipopolysaccharide (80 μg), as did Rylander et al, who found bronchoconstriction after inhalation of 200 μg lipopolysaccharide but not after 30 μg. These data suggest

Table 2 Comparison of the characteristics of the responders (R) and non-responders (non-R) to lipopolysaccharide

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>Non-R</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49 (11-3)</td>
<td>79 (13-8)</td>
<td>NS</td>
</tr>
<tr>
<td>M/F</td>
<td>3/5</td>
<td>6/2</td>
<td>NS</td>
</tr>
<tr>
<td>Smokers</td>
<td>2</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Total IgE (IU/ml)</td>
<td>629 (461)</td>
<td>404 (408)</td>
<td>NS</td>
</tr>
<tr>
<td>Positive skin test response</td>
<td>4</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>Atopic</td>
<td>5</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>Basal FEV, (% predicted)</td>
<td>89 (18-9)</td>
<td>90 (11-1)</td>
<td>NS</td>
</tr>
<tr>
<td>PD_{25}FEV, (μg)</td>
<td>257 (217)</td>
<td>723 (305)</td>
<td>0.035*</td>
</tr>
<tr>
<td>PD_{25}sGaw (μg)</td>
<td>81 (70-8)</td>
<td>368 (302)</td>
<td>0.027*</td>
</tr>
</tbody>
</table>

*p Mann-Whitney U test.

Abbreviations as in table 1.
that the response to lipopolysaccharide is dose related and that sensitivity to lipopolysaccharide is related to non-specific bronchial hyperreactivity, which may be a marker of bronchial inflammation. The correlation between the airway response to lipopolysaccharide and histamine in the present study supports this view.

The fall in FEV$_1$ by induced lipopolysaccharide was seen at 30 minutes, peaked at 60 minutes, and lasted more than five hours; this is consistent with the effect of induced lipopolysaccharide in patients with chronic bronchitis$^{19}$ or chronic non-specific lung disease with bronchial hyperreactivity.$^{20}$ The time course of the bronchial response to lipopolysaccharide is also consistent with cell recruitment into the airways after inhalation of endotoxin in animal models. In guinea pigs inhalation of endotoxin caused an influx of neutrophils in the airways at intervals ranging from 90 minutes$^{21,22}$ to four hours$^{23,24}$ and lasting at least 24 hours.$^{21,22}$ The fall in FEV$_1$ at 15 minutes was not significant. Absence of the early bronchoconstriction, seen in IgE mediated reactions, suggests that the response to lipopolysaccharide could be mediated by a non-IgE mechanism. This is supported by the presence of a bronchial response to lipopolysaccharide in some non-atopic subjects in the present study, by the absence of an immediate pricking skin test response to lipopolysaccharide in atopic subjects,$^{25}$ and by the absence of lipopolysaccharide induced histamine release by basophils from atopic subjects.$^{26}$

Studies in monozygotic and dizygotic twins$^{27}$ suggest that factors determining bronchial responsiveness are mainly exogenous rather than genetic. These may be IgE specific (sensitisation limited to the atopic subjects) or non-specific (in both atopic and non-atopic subjects). In our study the response to lipopolysaccharide correlated with non-specific bronchial responsiveness but was independent of the presence or absence of atopy. Lipopolysaccharide could be a factor of clinical relevance in both atopic and non-atopic bronchial diseases like intrinsic and extrinsic asthma, chronic bronchitis, and some occupational lung diseases.

We thank Mrs M J van Gyseghem for technical assistance and Mrs B van Bellegem for secretarial assistance.