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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% (PD_{40} 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 FEV_1 and area under the FEV_1-time curve.**

Results **FEV_1 fell significantly more after lipopolysaccharide than after diluent inhalation, the difference in mean (SE) FEV_1 being 4·6% (5·4%); response was maximal 60 minutes after lipopolysaccharide inhalation and lasted more than five hours. Histamine $PD_{20}FEV_1$ and PD_{40}sGaw correlated with the fall in FEV_1 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,[1] macrophages,[2 3] and mast cells[4]; they also activate several proinflammatory mediators, including complement,[5] arachidonic acid,[6] and neutrophil enzymes.[3] Lipopolysaccharides (the major part of endotoxin) have been found in commercial[7] and natural house dust extracts,[8] raising the possibility that they may cause problems in asthmatic patients.

In normal subjects inhalation of 200 μg lipopolysaccharide causes bronchoconstriction.[9] A dose of 20 μg causes no response in normal subjects[9 10] but causes bronchoconstriction in some asthmatic patients,[10] 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 PD_{40} 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.[11] Twelve patients had mild asthma treated with β_2 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 antihistamine, corticosteroid, methylxanthine, sodium cromoglycate, or non-steroid anti-inflammatory drugs. The asthmatic patients were asked to stop β_2 agonist drugs for 12 hours before each test. All subjects had baseline measurements of forced expiratory volume in one second (FEV_1) and airway resistance (Raw) within the normal range (that is, the predicted value ± 1·65 SD, as recommended by the Communauté Europeenne du Charbon et de l'Acier (CECA).[12]

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 diphosphate solu-

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Table 1 Characteristics of the subjects

Patient No	*Sex*	*Age (y)*	*Smoker*	*Atopic*	*Diagnosis*	*FEV_1 (% pred)**	*PD_{20} FEV_1 (μg histamine)*	*PD_{40} sGaw (μg histamine)*
1	M	40	+	+	Asthma	86	46·5	24
2	F	45	+	+	Asthma	72	123	40
3	F	38	+	−	Asthma	96	819	82
4	M	52	−	+	Asthma	100	900	465
5	M	63	−	−	Asthma	72	78	36
6	M	43	−	+	Asthma	115	529	248
7	M	65	−	−	Asthma	74	712	100
8	M	59	−	+	Asthma	77	111	48·5
9	F	33	−	+	Asthma	96	900	168
10	F	26	−	+	Asthma	90	198	93
11	F	38	−	+	Asthma	114	90	66
12	F	46	−	−	Asthma	90	108	35
13	F	26	−	−	Rhinitis	91	900	772
14	M	31	−	+	Rhinitis	90	900	360
15	M	32	−	−	Rhinitis	108	900	834
16	M	29	+	+	Rhinitis	106	529	228

*Basal FEV_1, measured on the day the histamine challenge test was carried out.
FEV_1—forced expiratory volume in one second; $PD_{20}FEV_1$—provocation dose of histamine causing a 20% fall in FEV_1; PD_{40}sGaw—provocation dose of histamine causing a 40% fall in specific airways conductance.

tion (Hal Products) in increasing concentrations (1, 2, 4, 8, 16, 32 mg/ml). Solutions were stored at 4°C and allowed to warm to room temperature one hour before use. The control solution was the histamine diluent (a saline 9 g/l solution containing phenol, Hal Products).

The lipopolysaccharide solution was prepared with an extract (obtained by the trichloroacetic acid method) of *E coli* serotype 026:B6 (lot 13 F–4019, Sigma) dissolved in a sterile saline 9 g/l solution, after this had been shaken (vortex for two minutes) to a final dilution of 1 mg/ml. Sterile saline (9 g/l) was used as a control.

All solutions were administered by a Mefar MB3 dosimeter, which includes a solenoid electrovalve delivering air from a compressor at 1·6 kg/cm^2 during inspiration. The nebuliser was programmed to open for 0·6 seconds, which produces an aerosol of 4 μl at each slow inhalation, starting from functional residual capacity to just below total lung capacity (TLC).

All measurements were made in a constant volume computerised body plethysmograph (Bodyscreen II, Jaeger). Raw, specific airway conductance (sGaw), and FEV_1 (best of three determinations) were obtained as described.[10]

HISTAMINE CHALLENGE

Five puffs of control solution were followed by increasing concentrations (1–32 mg/ml) of histamine solution. Each dose was given at three minute intervals; FEV_1 and sGaw were measured two minutes after each inhalation and expressed as percentages of the value after the control solution.[13] The cumulative provocative doses of histamine inducing a 20% fall in FEV_1 ($PD_{20}FEV_1$) or a 40% fall in sGaw (PD_{40}sGaw) 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. FEV_1 (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), FEV_1 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 FEV_1 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 χ^2 test with Yates's correction.[14]

The differences between values of FEV_1 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 FEV_1-time response (*area LPS 15–300*) measured by planimetry (60 minutes = 10% FEV_1). It was also measured as the mean decrease in FEV_1 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 FEV_1. When the result of the F test was significant, the difference in FEV_1 was compared with the baseline difference in FEV_1 by the modified *t* test with the Bonferroni correction.[15]

The significance of the lipopolysaccharide response within subjects was tested by a two way analysis of variance on the FEV_1 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.

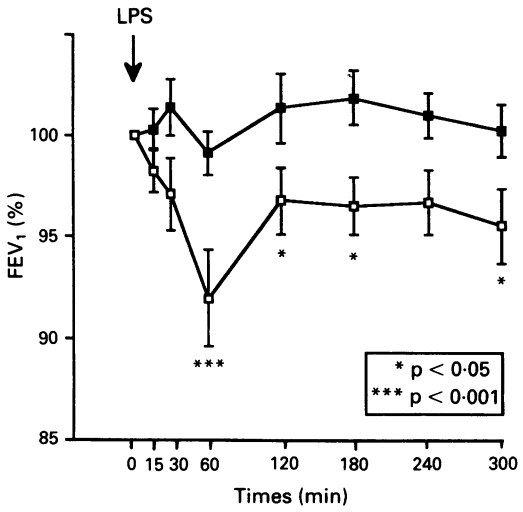

Figure 1 Mean (SE) results of the lipopolysaccharide (LPS) bronchial challenge test (□——□) compared with control results (■——■) in the 16 patients studied (F'_{105} = 3·139, p < 0·005; SE indicated by bars).

The relation between log $PD_{20}FEV_1$ and log PD_{40}sGaw for histamine and the area LPS 15–300 and LPS 15–300 was assessed by the linear correlation coefficient.[14] A p value below 0·05 was considered significant.

Results

The mean difference in change in FEV_1 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 $PD_{20}FEV_1$ (p < 0·02) and log PD_{40}sGaw (p < 0·001); r = −0·64 and −0·74 for area LPS 15–300 *v* log $PD_{20}FEV_1$ (p < 0·01) and log PD_{40}sGaw (p < 0·001)).

The group who responded to lipopolysaccharide (n = 8) had a mean fall in FEV_1 (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_1 or proportions of smokers and of atopic individuals (table 2). Responders to lipopolysaccharide had lower values than non-responders for $PD_{20}FEV_1$ (257 (217) *v* 723 (305) μg histamine; p = 0·025) and PD_{40}sGaw (81 (71) *v* 369 (302) μg histamine; p = 0·027).

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

	R	*Non-R*	*p*
n	8	8	
Age (years)	45·4 (11·3)	39 (13·8)	NS
M/F	3/5	6/2	NS
Smokers	2	2	NS
Total IgE (IU/ml)	629 (461)	404 (408)	NS
Positive skin test response	4	5	NS
Atopic	5	5	NS
Basal FEV_1 (% predicted)	89·5 (18·9)	93·9 (11·1)	NS
PD_{20} FEV_1 (μg)	257·0 (271·2)	723·3 (305·5)	0·025*
PD_{40} sGaw (μg)	81·1 (70·8)	368·8 (302·1)	0·027*

*Mann-Witney U test.
Abbreviations as in table 1.

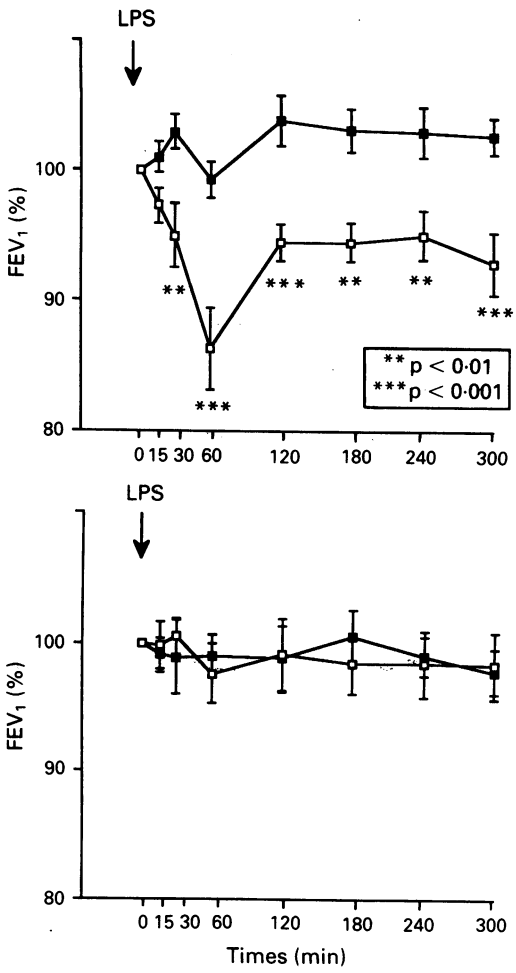

Figure 2 Results of the lipopolysaccharide (LPS) bronchial challenge test (□——□) compared with control results (■——■) in responders (top panel) and non-responders (bottom panel) to lipopolysaccharide; FEV_1 is expressed as a percentage of the basal value. A two way analysis of variance was applied (F'_{49} = 6·39, p < 0·001, and 0·44, NS; SE indicated by bars) and p values were calculated with Bonferroni correction.

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[1–3 6 16–18] and man.[10 19]

There was a wide range of response to lipopolysaccharide amongst the 16 subjects, so the group was divided into lipopolysaccharide responders and non-responders. The main characteristic of the subjects who produced a significant fall in FEV_1 after inhaling 20 μg lipopolysaccharide was their greater histamine bronchial responsiveness. This is consistent with studies of van der Zwan *et al*,[20] who showed that inhalation of *Haemophilus influenzae* endotoxin induced decrease in FEV_1 only in patients with histamine hyperresponsiveness. In the study of Cavagna *et al*[19] 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*,[9] who found bronchoconstriction after inhalation of 200 μg lipopolysaccharide but not after 30 μg. These data suggest

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 induced by lipopolysaccharide was seen at 30 minutes, peaked at 60 minutes, and lasted more than five hours; this is consistent with the effect of inhaled 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[3 21 22] to four hours[18] and lasting at least 24 hours.[3 18 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 prick skin test response to lipopolysaccharide in atopic subjects,[23] and by the absence of lipopolysaccharide induced histamine release by basophils from atopic subjects.[24]

Studies in monozygotic and dizygotic twins[25] 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.

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