

Healthy subjects express differences in clinical responses to inhaled lipopolysaccharide that are related with inflammation and with atopy

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Background: Endotoxin and its purified derivative LPS are important contaminants of both domestic and occupational environments that have been related to airway diseases. A body of data suggests that there is considerable interindividual variability in LPS sensitivity.

Objective: The aim of the study was to relate the individual clinical responses to inhaled LPS with the inflammatory process and the atopic status.

Methods: Fifteen healthy subjects were challenged each week by inhalation with saline solution or LPS (0.5, 5, or 50 µg). The systemic response was defined by the increase in body temperature, blood neutrophilia, acute-phase proteins (C-reactive protein and LPS-binding protein [LBP]), and E-selectin. The LPS-induced airway response was defined as the increase in airway responsiveness and related to the cell count and concentration of TNF-α, myeloperoxidase, and eosinophil cationic protein in induced sputum. The atopic status was defined as an increase in IgE or a positive skin prick test result.

Results: Subjects (n = 7) with a significant increase in body temperature had a larger increase in the systemic inflammatory response (blood neutrophilia; $P < .01$) and in blood concentrations of C-reactive protein ($P < .02$) and LBP ($P < .01$). Subjects with a significant increase in airway responsiveness (n = 8) had an increase in the sputum concentration of eosinophil cationic protein ($P < .01$). The amplitude of the systemic response (increase in body temperature [$P < .001$], blood neutrophilia [$P < .02$], and rise in LBP [$P < .05$] and decrease in FEV₁ [$P < .01$]) were inversely associated with the atopic status, suggesting a link between atopy and LPS responsiveness.

Conclusions: The clinical response to LPS occurs systemically or locally and is associated with inflammation. The atopic status was inversely related to the systemic inflammation.

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Key words: Endotoxin, fever, airway responsiveness, inflammation, atopy

Endotoxin and its purified derivative LPS are gram-negative bacterial constituents continuously shed into the environment.¹ A number of different gram-negative bacteria inhabit the normal body surfaces including the skin, oral cavity, respiratory tract, gastrointestinal tract, vagina, and urinary tract. In addition to the septic shock frequently caused by translocation of gram-negative microorganisms normally present in the gut of the host to the circulation, there is continuous exposure to airborne endotoxin. The release of endotoxin from gram-negative bacteria, which colonize the respiratory tract in most patients with chronic bronchitis, can contribute to the lung function decrease by initiating release of inflammatory mediators from bronchial epithelial cells.² High levels of airborne (1 µg/m³) endotoxin have been reported from a variety of occupational environments (eg, swine pens, poultry farms, cotton mills, breweries, waste processing plants).¹ A number of cross-sectional studies report an association between endotoxin exposure in occupational settings and the risk of developing nonatopic, chronic obstructive pulmonary diseases,³⁻⁶ toxic pneumonitis, and systemic effects.^{7,8} In the domestic environment, there is also endotoxin contaminating house dust⁹⁻¹² that could be an important determinant of asthma severity.^{9,10}

Sensitivity to endotoxin varies considerably among animal species, with human subjects being very sensitive. In healthy human subjects, inhalation of endotoxin induces dose-related clinical symptoms, decrease in lung function, and bronchial as well as systemic inflammatory responses.¹¹ There is considerable interindividual variability in the amplitude of both the clinical and inflammatory responses to purified LPS by inhalation^{3,13-15} or by intravenous administration.¹⁶ In mice, endotoxin sensitivity is genetically determined,¹⁷ involving mutation in the Toll-like receptor-4 gene,¹⁸ a coreceptor essential for LPS signaling. In human beings recent data suggest that polymorphisms in the genes encoding the proinflammatory LPS-induced cytokine TNF-α¹⁹ or the LPS receptor CD-14²⁰ may be related to symptoms and diseases. Activation of antigen-presenting cells through LPS receptor CD14 causes the release of several cytokines that modulate the specific immune

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Abbreviations used

AR:	Airway responsiveness
BPI:	Bactericidal/permeability increasing protein
CRP:	C-reactive protein
DL:	Detection limit
ECP:	Eosinophil cationic protein
LBP:	LPS-binding protein
MPO:	Myeloperoxidase
PME:	Polymorphonuclear eosinophil
sE-selectin:	Soluble E-selectin
sICAM:	Soluble intercellular adhesion molecule
sTNF-R:	TNF soluble receptor

response, inhibiting the production of IgE.²¹ Therefore the responsiveness to LPS could interfere with the development of allergic diseases.

The aim of this study was to determine the effects of inhaled LPS in healthy subjects in terms of the systemic and the local responses. The relationship between the response to LPS and the atopic status was also evaluated.

METHODS

Subjects

A group of 15 healthy, asymptomatic, nonsmoking subjects, preselected for their ability to produce an adequate sample of induced sputum, were included. The mean age was 31.1 (± 3.0) years; 8 were female and 7 male. Total and specific blood IgE levels (CAP system; Pharmacia, Uppsala, Sweden) for airborne allergens were determined. On the basis of positive (≥ 4 -mm wheal) skin prick test results for the most frequent allergens (Bencard, Worthing, UK) and/or positive specific IgE for at least 1 allergen,²² 9 subjects were considered atopic, and 6 were nonatopic (Table I).

The protocol was approved by the ethical committee of the hospital, and written, informed consent was obtained.

General study design

All of the nebulized solutions were administered by a Mefar dosimeter MB3 (Mefar, Brescia, Italy) as previously reported.²³ By use of a single-blind procedure, all the subjects were challenged each week by inhalation of either sterile apyrogen saline solution or a dose (0.5, 5, and 50 μ g) of a suspension of LPS (*Escherichia coli* 026:B6, reference No. L-2654, lot 43H40491; Sigma Chemical, St Louis, Mo) in the same order. The 0.5- μ g dose corresponds to the no-response threshold, whereas 50 μ g is associated with symptoms in some subjects.^{13,23,24}

Fever and lung function were recorded before and at 1, 6, 8, and 24 hours after specific airway responsiveness (AR) was measured, 8 hours after the challenge. Induced sputum was obtained at 6 hours. Blood samples were collected at 0, 6, and 24 hours.

Lung function

Lung function was measured by plethysmography (Bodyscreen II; Jaeger, Würzburg, Germany). Usually AR is evaluated by the cumulative dose of histamine inducing a 20% fall in FEV₁.²³ However, because our healthy subjects did not achieve a decrease of 20% in FEV₁, the results were expressed by their maximal decrease in FEV₁ (as a percentage of the baseline value) measured after the highest dose of histamine (900- μ g cumulative dose).

Induced sputum

Induced sputum was collected with the method described by Pin et al²⁵ adapted for healthy subjects.¹³ The hypertonic sterile saline solution (5%) was nebulized for 30 minutes with an ultrasonic nebulizer (Fisoneb; Karapharm, Marseille, France); subjects were asked to rinse their mouths with water every 5 minutes and to try to cough sputum directly into a sterile plastic box. The sputum was weighed, mixed with an equal volume of dithiothreitol 10% (Sputolysin; Behring Diagnostics, Somerville, NJ) and incubated for 15 minutes at 37°C. After centrifugation (15 minutes at 800g) the supernatant was frozen at -20°C while the pelleted cells were washed 2 times and resuspended in PBS. The number of cells was measured with a Thoma's hemocytometer. A slide was prepared by centrifugation (Cytospin, Shandon Inc, Pittsburgh, Pa) and stained with May-Grünwald-Giemsa before the differential cells were counted by 2 independent experienced readers. The differential cell count was consistent with data from another study using a similar method.²⁴

The TNF- α was measured in the supernatants with a specific immunoenzymatic assay (Medgenix Diagnostics, Fleurus, Belgium), whereas eosinophil cationic protein (ECP) and myeloperoxidase (MPO) were measured with RIA commercial kits (Pharmacia, Uppsala, Sweden).¹³

Blood markers of the response to LPS

An automated method (H1 counter; Technicon Instruments, Tarrytown, NY) was used to count the total and differential white blood cells.

Several blood markers of endotoxin activation were evaluated: the soluble E-selectin (sE-selectin),²⁶ the soluble intercellular adhesion molecule (sICAM-1),²⁶ the 2 soluble forms of TNF receptors (sTNF-R55 and sTNF-R75),²⁷ the bactericidal/permeability-increasing protein (BPI),²⁸ and the acute-phase C-reactive protein (CRP) and LPS-binding protein.²⁹

The blood CRP was measured with a 2-site monoclonal antibody enzyme-linked immunoassay (antibodies and calibrator from Dako Ltd, High Wycombe, UK; detection limit (DL), 0.5 mg/L).¹³ Plasma sTNF-R55, sTNF-R75, LBP, BPI, sICAM-1, and sE-selectin, were measured with sandwich ELISA as reported previously.³⁰⁻³⁴ For measurement of sTNF-R55 and TNF-R75 monoclonal antibodies, MR1-1 and MR2-2 were used for coating. Specific biotin-labeled polyclonal rabbit-antihuman sTNF-R IgG was used as a detector reagents, and the standards were recombinant human sTNF-R55 and sTNF-R75 (DL, 100 pg/mL). Polyclonal rabbit anti-rh LBP IgG was used as coating for the LBP ELISA, and biotin-labeled polyclonal rabbit anti-human recombinant LBP was used for detection; the standard was human recombinant LBP (DL, 200 pg/mL). For measurement of BPI, plates were coated with monoclonal antibody 4E3, and BPI was detected by polyclonal rabbit anti-human BPI antiserum (DL, 200 pg/mL). For sICAM-1 ELISA, mAb HM.2 was used for coating, recombinant human ICAM-1 was used as a standard, and biotinylated mAb HM.1 was used for detection (DL, 400 pg/mL). For sE-selectin ELISA, plates were coated with mAb ENA1, and biotin-labeled mAb ENA2 was used as the detector reagent; the standard was recombinant human sE-selectin (DL, 1 ng/mL).

Definition of clinical response to inhaled LPS

The clinical responses to inhaled LPS was defined by objective parameters: a change in temperature, a change in AR, or a change in FEV₁. A significant change in temperature was defined as an increase of more than 0.5°C after LPS compared with after saline solution. A significant change in AR was defined as an increase in AR ($>10\%$ histamine-induced fall in FEV₁ compared with after saline solution), and a significant change in lung function was defined as a significant decrease in FEV₁ (see below).

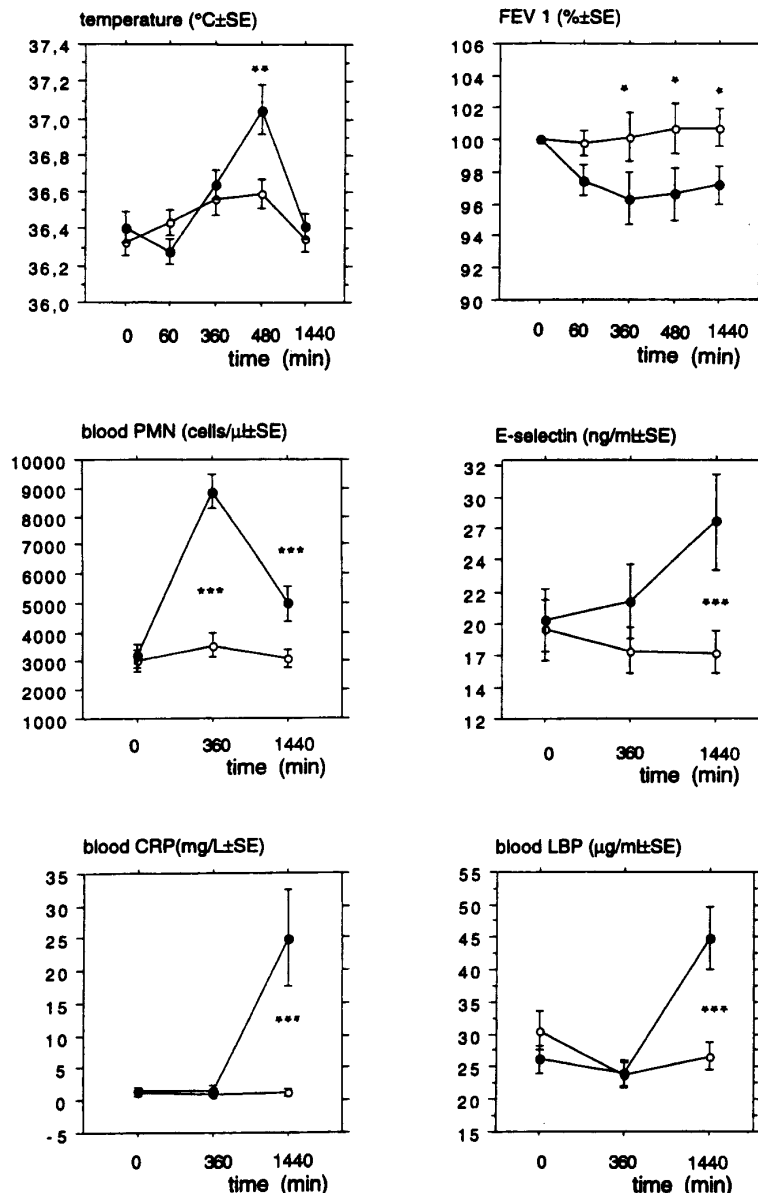


FIG 1. The time-related response induced by inhalation of 50 µg LPS (●) compared with saline solution (○). Results are means (±SE) of the changes in temperature (F test = 4.9, $P < .01$), FEV₁ (F test = 3.1, $P < .05$), blood PMN count (F test = 55.3, $P < .001$), blood concentration in E-selectin (F test = 9.9, $P < .001$), CRP (F test = 11.9, $P < .001$), and LBP (F test = 24.5, $P < .001$). Wilcoxon paired test: * $P < .05$, ** $P < .02$, *** $P < .01$.

Statistical analysis

All the results were expressed as mean ± SE. The response in FEV₁ was calculated as the change in the percentage of the basal value. The significance of the FEV₁ response within subjects was tested by 2-way ANOVA (factors: time and challenge). A patient was defined as having a significant FEV₁ response according to whether the result of the F test was significant. Blood and sputum parameters were expressed in absolute values, and their changes after LPS were compared with those after saline solution. A 2-way ANOVA was performed on each parameter (factors: subject and either time or challenge).³⁵ When the F test results were significant, paired or nonpaired values were tested by the Wilcoxon or Mann-Whitney nonparametric tests, respectively. A P value $< .05$ was considered as significant.

RESULTS

The time-related response to 50 µg LPS was analyzed to define the time at which body temperature, lung function, and blood inflammatory indicators were at maximum (Fig 1). A significant decrease in FEV₁ was found 1 hour after 50 µg LPS and lasted more than 8 hours. The LPS-induced increase in neutrophilia and temperature peaked at 6 and 8 hours, respectively, whereas the peaks of CRP, LBP, and sE-selectin occurred at 24 hours. No significant changes were present for the blood sTNF-R55, sTNF-R75, sICAM, or BPI. Flu-like symptoms developed in 4 subjects.

TABLE I. Subjects characteristics and atopic status

Subject No.	Age (y)	Sex	C	H	SPT (≥4 mm)	Specific IgE (>1 U/mL)	IgE (U/mL)	Atopic status
1	24	M	0	3	+ (Dpt)	–	14	Atopic
2	25	M	0	5	–	+ (Dpt)	36	Atopic
3	45	F	0	4	–	–	15	Nonatopic
4	45	F	0	3	–	–	24	Nonatopic
5	43	F	0	3	–	–	29	Nonatopic
6	24	M	0	4	+ (Dpt, cat, grass)	+ (Dpt, grass)	305	Atopic
7	28	M	0	3	+ (grass)	+ (grass)	239	Atopic
8	31	M	0	3	–	–	30	Nonatopic
9	50	F	0	3	+ (Dpt, cat, tree)	+ (Dpt)	92	Atopic
10	22	M	0	5	+ (Dpt, cat, grass)	+ (Dpt, cat, grass)	1790	Atopic
11	18	F	0	5	–	–	5	Nonatopic
12	20	M	0	5	+ (cat, grass, Asp, Herb)	+ (grass)	65	Atopic
13	49	F	0	6	+ (grass, Alt)	–	24	Atopic
14	23	F	0	4	+ (grass)	–	6	Atopic
15	19	F	0	3	–	–	96	Nonatopic

C, Control SPT; H, histamine SPT; Dpt, house dust mite; Alt, *Alternaria*, Asp, *Aspergillus*; Herb, *Herbacea*.
Atopic status is defined by positivity for SPT and/or for specific IgE.

TABLE II. The basal values of responders and nonresponders to LPS

	Change in temperature			Change in AR		
	R	Non-R	P	R	Non-R	P
Sex (F/M)	6/1	2/6	<.06*	4/4	4/3	NS*
Atopy (yes/no)	1/6	5/3	NS*	3/5	3/4	NS*
Age, y ($\pm$ SE)	35.4 (5.1)	27.2 (3.3)	NS	24.8 (2.9)	38.3 (4.4)	<.02
Weight, kg ($\pm$ SE)	66.4 (3.8)	73.2 (3.9)	NS	68.0 (4.3)	72.4 (3.5)	NS
AR (% FEV ₁ change)	-9.9 (7.2)	-12.6 (9.0)	NS	-12.0 (5.5)	-10.6 (10.7)	NS
Sputum total cells $\times 1000/\text{mL}$ ($\pm$ SE)	1091 (428)	2082 (303)	<.05	1700 (360)	1528 (248)	NS
E-selectin, ng/mL ($\pm$ SE)	14.7 (1.6)	22.8 (3.8)	NS	16.3 (2.3)	22.2 (4.2)	NS
CRP, mg/L ($\pm$ SE)	1.3 (0.4)	1.2 (0.5)	NS	0.4 (0.2)	2.2 (0.4)	<.001
LBP, $\mu\text{g/mL}$ ($\pm$ SE)	31.8 (4.4)	29.6 (4.1)	NS	25.3 (3.4)	36.7 (3.9)	<.05
BPI, ng/mL ($\pm$ SE)	18.1 (5.2)	11.1 (2.5)	NS	13.2 (4.2)	15.8 (3.9)	NS
ICAM, ng/mL ($\pm$ SE)	90.0 (9.2)	103.6 (17.6)	NS	96.4 (18.0)	98.3 (9.1)	NS
TNF-R55, ng/mL ($\pm$ SE)	0.9 (0.1)	0.8 (0.1)	NS	0.9 (0.1)	0.9 (0.1)	NS
TNF-R75, ng/mL ($\pm$ SE)	2.1 (0.3)	1.8 (0.1)	NS	1.9 (0.1)	2.0 (0.3)	NS

R, Responders; Non-R, nonresponders; NS, nonsignificant.

Statistics: Mann-Whitney *U* test or χ^2 test with Yates correction.

The responses in body temperature and in blood neutrophilia, sE-selectin, CRP, and LBP were dose related. In the airways the number of PMN, lymphocyte, and inflammatory mediators (MPO, TNF- α , ECP) rose significantly, the amplitude of the response being dose related. There was no significant change in the sputum polymorphonuclear eosinophil (PME) count.

Subjects with an increase in AR also had a significant increase in sputum ECP (not in the PME count) (Fig 2). The changes in sputum cells, TNF- α , MPO, and blood inflammatory markers were not significantly different. Subjects who did not have increases in AR had higher pre-exposure blood levels of CRP and LBP, although they were not different regarding atopic status, sputum cytology, and blood inflammatory markers (Table II).

FEV₁ decrease was not significantly associated with the change in blood markers of inflammation or with the change in AR. There was a trend, although not significant, between

the FEV₁ response and the sputum ECP and MPO (Fig 2).

An LPS-induced body temperature increase was more frequent in women than in men, whereas age, weight, and basal blood inflammatory marker sputum cells were not significantly different compared with those of the nonresponders (Table II). Subjects with increased body temperature had a significantly larger rise in blood CRP, LBP, and neutrophilia (Fig 2), although the LPS-induced changes in sputum were not associated with the body temperature increase.

The amplitudes of the systemic responses (temperature, neutrophilia, and rise in LBP but not CRP) as well as the FEV₁ response were significantly larger in the nonatopic (*n* = 6) than in the atopic (*n* = 9) subjects, whether atopy was defined as increased specific IgE or positive SPT (Fig 3). In contrast, the airway inflammatory response (sputum TNF, ECP, MPO, and cell count) was not associated with the atopic status (Fig 3 and Table III).

CLINICAL RESPONSES TO INHALED LPS

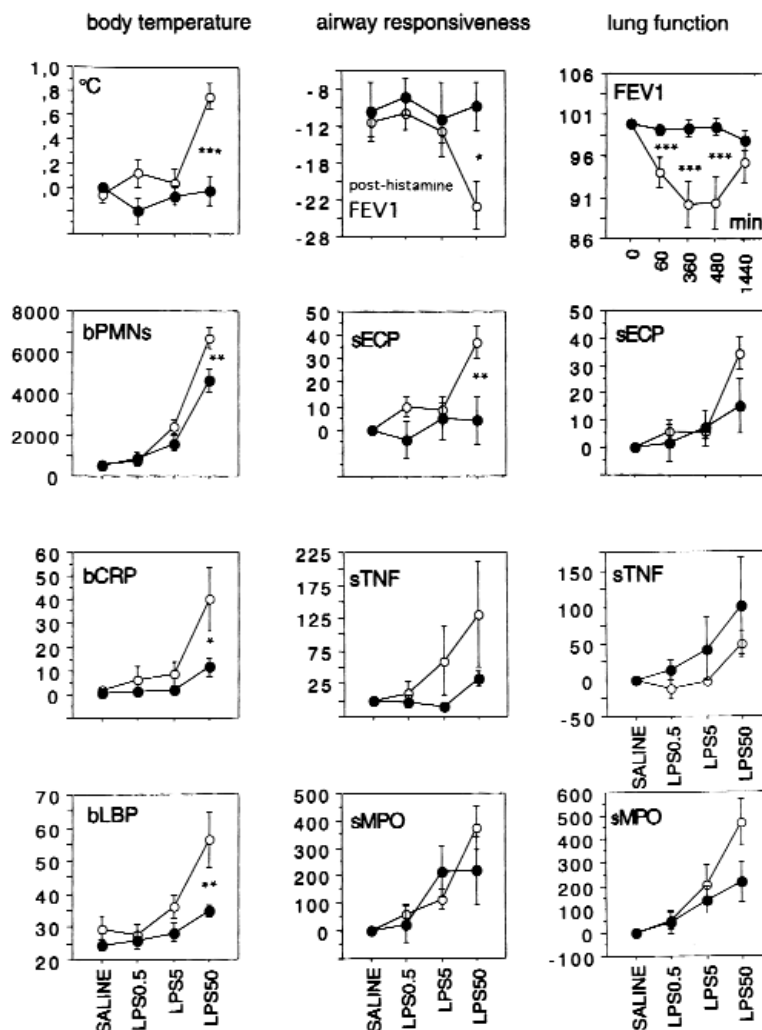


FIG 2. The association between the clinical responses to inhaled LPS with inflammation. **Left,** Compared with nonresponders (●), subjects who had a significant increase in body temperature (○; F test = 16.3, $P < .01$) also had larger responses in blood inflammatory markers: PMN (*bPMN*; F test = 7.6, $P < .01$), CRP (*bCRP*; F test = 6.9, $P < .02$), and LBP (*bLBP*; F test = 11.0, $P < .01$). **Center,** Compared with nonresponders (●), subjects with a significant increase in AR (○; F test = 4.5, $P < .05$) also had a significant rise in sputum ECP (*sECP*; F test = 8.4, $P < .01$), but not in sputum TNF (*sTNF*) or sputum MPO (*sMPO*). **Right,** Compared with nonresponders (●), subjects with a significant decrease in FEV₁ (○; F test = 31.2, $P < .001$) were not significantly associated with the response in sputum ECP (F test = 1.1, $P > .1$), TNF (F test = 2.9, $P = .09$), or MPO (F test = 3.0, $P = .09$). Mann-Whitney tests: * $P < .05$, ** $P < .02$, *** $P < .01$. PMNs are expressed in cells per microliter, CRP in milligrams per liter, LBP in milligrams per liter, FEV₁ as a percentage, ECP in nanograms per milliliter, TNF in picograms per milliliter, and MPO in nanograms per milliliter. All data are means $\pm$ SE.

DISCUSSION

We have shown that healthy subjects exposed to LPS express differences in changes in body temperature, AR, and FEV₁. Subjects who had an increase in temperature showed an increase in indicators of inflammation in the blood (systemic response). Those who had an increase in AR showed an increase in indicators of airway inflammation (local response) but not in the blood. Those who had a decrease in FEV₁ did not show an increase in inflammatory markers. The atopic status was inversely related to the systemic LPS response.

The kinetics of the response to inhaled LPS or LPS-contaminated dust have been reported in several studies. The onset of the decrease in FEV₁ occurs at 1 hour,^{3-5,11,15,23} the rise in blood neutrophils at 4 to 6 hours,^{13,15} the onset of fever and symptoms at 8 to 12 hours,¹³⁻¹⁵ and a peak in CRP at 24 to 48 hours.¹³ In this study, new findings included increases in blood sE-selectin and LBP at 24 hours, whereas the sICAM-1 adhesion molecule did not change significantly. E-selectin is expressed by bronchial endothelial cells after only a few hours,^{26,30} suggesting that it could contribute to the following

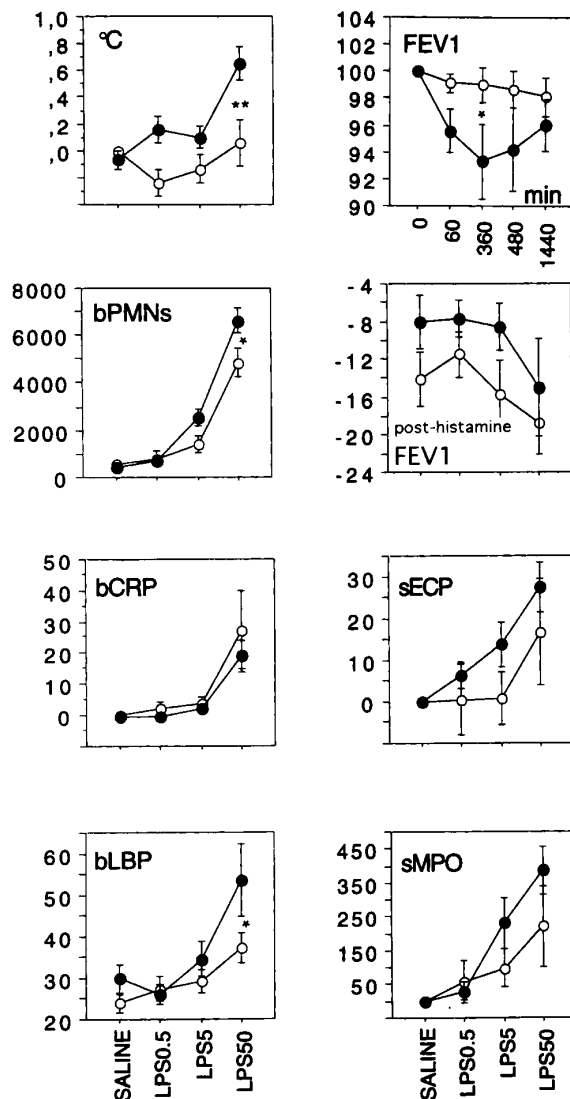


FIG 3. The association between the clinical and inflammatory responses to inhaled LPS with atopy (ie, SPT ≥ 4 mm). **Left.** Compared with atopic subjects (○), the amplitude of the systemic response is larger in nonatopic subjects (●): increase in body temperature ($^{\circ}\text{C}$; F test = 14.8, $P < .001$), blood PMN (bPMNs; F test = 6.9, $P < .01$), and blood LBP (bLBP; F test = 5.2, $P < .03$), but not blood CRP (bCRP). **Right.** Nonatopic subjects (●) had a larger decrease in FEV₁ (F test = 8.9, $P < .01$; upper panel) but not in AR, sputum ECP (sECP), or sputum MPO (sMPO) (lower 3 panels). Mann-Whitney tests: * $P < .05$, ** $P < .02$. PMNs are expressed in cells per microliter, CRP in milligrams per liter, LBP in milligrams per liter, FEV₁ as a percentage, ECP in nanograms per milliliter, TNF in picograms per milliliter, and MPO in nanograms per milliliter. All data are means $\pm$ SE.

recruitment of neutrophils reported 24 hours after inhalation of 40 or 60 μg LPS.^{13,24} In this study the rise in blood and airway neutrophils preceded the increase in blood concentration of sE-selectin, suggesting the participation of other chemotactic factors. The rise in the acute-phase proteins LBP and CRP suggests that hepatocytes are activated. This is probably because of the

release of IL-1, IL-6, and TNF- α from the LPS-activated airway macrophages.³⁶ As LBP participates in the LPS responsiveness, the rise of LBP concentration could modulate the response to LPS.^{29,37}

As reported, in healthy subjects the response to inhaled LPS is dose related.¹³ Airway neutrophils, lymphocytes, macrophages, and eosinophils participate in the LPS response. The rise in blood E-selectin suggests that the endothelial cells also participate in the LPS response. The LBP and E-selectin blood levels were sensitive markers of LPS exposure because they were significantly elevated after 5 μg inhaled LPS; this exposure level corresponds to that of a subject exposed for 10 hours per day and ventilating 1 m^3/h air containing 500 ng LPS, a level often found in occupational settings.^{1,3,5,6,8} The blood LBP and/or E-selectin could thus be used as markers of LPS exposure in field studies.

The LPS-induced increase in AR was associated with a significant rise of sputum ECP in the absence of a significant change in the number of eosinophils, suggesting a release of ECP from activated PME in sputum or from PME infiltrating airway tissue but not the airways.

Consistent with data from animal models, the LPS-induced increase in AR was dissociated from lung TNF- α release and neutrophil sequestration.³⁸ Because the lung neutrophil migration is not necessarily associated with an increase in metabolic activity,³⁹ LPS inhalation could induce airway infiltration of nonactivated neutrophils without clinical consequences, whereas ECP release from resident airway eosinophils could be involved in the change of AR.

The basal blood levels in CRP and LBP were higher in subjects who did not have an increase in AR after LPS. Some data suggest that CRP has anti-inflammatory properties that could diminish lung injury by a reduction in neutrophil influx⁴⁰; however, there was no relation between the blood level of CRP measured before LPS challenge and the airway neutrophilic response.

Subjects who had an increase in temperature had a larger response to inflammatory markers in blood without a relation to the airway inflammatory response, whereas their basal characteristics were not significantly different.

The LPS-induced increase in temperature and change in AR were not associated in a given subject, suggesting that the underlying mechanisms are dissociated. Therefore LPS sensitivity in healthy subjects can be defined in 2 ways: the systemic response to temperature increase and the local response to change in AR. Both types of effects are associated with inflammation in their respective compartments.

The LPS-induced lung function response may be modulated by environmental factors. In patients with asthma, the LPS-induced bronchoconstriction is not related to the atopic status, but it is significantly associated with basal nonspecific AR.^{15,41} The latter may be modulated by several environmental factors such as virus, ozone, and smoking.^{42,43} Moreover, in atopic subjects, pre-exposure to allergens enhances the LPS-induced airway inflamma-

TABLE III. Response to 50- μ g LPS compared with saline solution, in regard to atopic status

	SPT					
Response to LPS	≥4 mm	<4 mm	P	Atopic*	Nonatopic*	P
Systemic						
Temperature (°C)	0.06 (0.17)	0.66 (0.12)	<.02	0.10 (0.18)	0.61 (0.14)	<.05
PMN (cells/μL)	4825 (600)	6600 (540)	<.05	4670 (560)	6780 (500)	<.02
LBP (μg/mL)	34.2 (3.4)	53.5 (8.7)	<.05	34.1 (3.4)	53.6 (8.7)	<.05
CRP (μg/mL)	28.8 (13.9)	20.6 (4.6)	NS	27.1 (14.2)	22.5 (3.9)	NS
E-selectin (ng/mL)	28 (4.5)	27 (6.8)	NS	34.0 (6.11)	20.1 (2.7)	NS
Airway						
AR (%)	18.8 (3.2)	15.0 (5.1)	NS	20.5 (2.8)	13.0 (5.1)	NS
FEV ₁ (%)	99.0 (0.7)	95.8 (1.6)	NS	98.9 (0.7)	96.0 (1.7)	NS
Sputum lymphocytes (%)	5.4 (1.2)	6.9 (1.6)	NS	5.0 (1.2)	7.3 (1.5)	NS
Sputum PMN (%)	46.8 (5.8)	54.0 (6.9)	NS	45.4 (55.6)	55.6 (6.8)	NS
Sputum PME (%)	0.4 (0.3)	3.0 (1.8)	NS	1.8 (1.4)	1.4 (1.3)	NS
ECP (ng/mL)	16.7 (12.9)	27.4 (5.9)	NS	17.5 (13.0)	26.6 (5.8)	NS
TNF (pg/mL)	125.0 (81)	37.8 (12.6)	NS	46.0 (15)	128.0 (93)	NS
MPO (ng/mL)	221 (119)	387 (71)	NS	188 (111)	425 (68)	NS

AR, Airway responsiveness; NS, nonsignificant.

*Atopic subjects are defined as testing positive for SPT and/or specific allergen.

tion.²⁹ This suggests that the LPS-induced changes in AR and lung function vary with exogenous environmental conditions, whereas the systemic response (fever and change in blood inflammatory markers) is more related to the endogenous LPS sensitivity.

An important mechanism for LPS effect is binding to the macrophage surface through the CD14 receptor³⁷; LPS can also activate the antigen-presenting dendritic cells through a soluble CD14 (sCD14)-dependent pathway.⁴⁴ The dendritic cells are key cells in the control of the primary immune response in early childhood. The LPS stimulation of dendritic cells results in production of IL-12, a major cytokine for differentiation of naive CD4⁺ T cells into the T_H1 type.⁴⁵ A defect in this mechanism could result in the persistence of T_H2 cells, secreting IL-4 and switching the B cell into IgE production and atopic status. Recently, a polymorphism in the CD14 gene has been reported, in terms of C:T substitution in the flanking region of the gene.²⁰ The homozygotes for the C allele (C:C) and heterozygote (C:T) were associated with low sCD14 and high IgE levels. The sCD14 level was negatively correlated with IL-4 and positively correlated with IFN- γ responses to mitogens by blood monocytes,²⁰ suggesting that genetic influences may control circulating levels of CD14 and, by this mechanism, may modulate the T_H1/T_H2 balance and IgE synthesis.⁴⁶

This study has shown that atopic subjects are less prone to mount a systemic response (ie, fever, neutrophilia, and acute-phase protein) to inhaled LPS than nonatopic subjects. This observation reinforces the hypothesis for a mechanism linking the macrophage susceptibility to LPS stimulation with the increase in macrophage production of cytokines that inhibits the T_H2 response and consequently the risk to become atopic. This hypothesis has previously been proposed by Holt et al⁴⁷: environmental exposure to LPS and other bacterial wall products present in house dust^{9,10} and/or the intesti-

nal tract⁴⁸ could be a necessary step for maturation of the immune system and the development of a T_H1-like response through the presentation of antigen in conjunction with IL-12. To our knowledge, the present results are the first in vivo data from human beings that associate the atopic status with the LPS response. If this is mediated through CD14, genetic variation in the CD14 gene could contribute to both the LPS response and the risk of atopy.

In conclusion, we found that human subjects express local as well as systemic responses when exposed to LPS by inhalation. Both responses are associated with inflammation but only at their respective sites. The LPS-induced temperature increase is related to the blood inflammation and inversely to the atopic status. Reaction patterns to inhaled LPS may provide information about the link between innate and adaptive immunity.

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