THE JANET FISCHER LECTURE
ENDOTOXIN INHALATION CHALLENGES IN HUMANS

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Introduction

An inhalation-challenge study is defined as a test in which the subject is experimentally exposed to an agent that elicits a clinical and/or biological response, assesssed by objective measurements. In contrast to the difficulties of evaluating all factors that may modify the response in the general environment or workplace setting, challenge studies allow control of the exposure and the subsequent effects.

Inhalation techniques vary greatly. Airway and lung exposure can be achieved by inhalation of the pure endotoxin suspended in solvent after nebulization in small aerosol particles to optimize deposition below the larynx. In the second method, the exposure corresponds to inhalation of endotoxin-containing dust particles; subjects are placed in an experimental room equipped to mimic their occupational activity. Several investigators have also reported the responses to inhalation of extracts of endotoxin-containing dusts.

The response endpoints used to evaluate effects of acute endotoxin inhalation include symptoms and clinical findings, lung function, blood inflammatory markers and cell count, airway inflammation (broncho-alveolar lavage, induced sputum, bronchial biopsies).

Responses to inhaled endotoxin

After inhalation of pure endotoxin (30-300 µg), some normal subjects experience short periods (less than four hours) of fever and tightness of the chest six to eight hours after exposure (1), while a dose of less than 20 µg does not induce symptoms (2,3,4). We have reported that a dose of 50 µg may induce fever, myalgia, and malaise in about 50% of normal subjects (5) and, in a recent study, symptoms were induced by 40 g (6). One inhaled dose higher than 80-200 g of pure endotoxin is required to produce moderate acute decrement in FEV1 in normal subjects (7) while a lower dose (12-50 µg) of aerosolized endotoxin solution had no effect on lung function as measured by FEV1, airway resistance, or conductance (1-7).

There are also data on effects of endotoxin in patients with chronic obstructive pulmonary diseases. In slight asthmatics, an acute inhalation of 20 µg of aerosolized pure endotoxin induces a lung function response, characterized by bronchoconstriction and change in the level of non specific bronchial hyperresponsiveness (BHR)(2,7,8). This endotoxin induced FEV1 decrease is associated, on one side, with a similar response in FVC (forced vital capacity), the FEV1/FVC being unchanged, suggesting a restrictive functional defect and, on the other side, with a rise in airways resistances indicating an obstructive component in the final response (see figure). In asthmatics, the endotoxin-induced bronchoconstriction is related to the individual level of non-specific BHR but not with atopy, suggesting that pre-existing non specific airways inflammation is a factor of sensitivity to inhaled endotoxin (9).

Inhalation of dust contaminated by endotoxin produces significant clinical and lung function response. Grain handlers exposed to 30 µg endotoxin in the dust experience fever, tachycardia and changes in respiratory rate (10). Larsson et al. showed that the level of exposure-inducing symptoms in normal subjects was 1.8 to 3.0 µg endotoxin measured in airborne swine dust (11). After 6 hours exposure to endotoxin contaminating cotton dust in pre-selected healthy subjects and cotton workers, Castellan et al.(12) and Rylander et al. (13) reported a threshold value for significant change in FEV_1 of approximatively 50 ng/m³.

Local and systemic inflammatory responses have been measured after endotoxin inhalation. Significant blood leukocytosis and neutrophilia were observed 4 to 8 hours after 20 µg inhaled endotoxin (3,5,8,11); this response was not related with the lung function. However the amplitude of the endotoxin-induced fever seems associated with the neutrophilia (unpublished). In normal subjects, 100 µg of inhaled pure endotoxin induced a 100-fold increase in neutrophils from bronchoalveolar lavage (BAL), associated with a rise in fibronectin (14). Compared to the saline, a neutrophilic infiltration in sputum was significant after 5 µg endotoxin inhalation and this response was significantly reinforced after the highest tested dose (50 µg) of inhaled endotoxin, demonstrating a dose-response relationship (5). Airways inflammation, characterized by neutrophil recruitment in BAL was also found after bronchial challenges with endotoxin-contaminated dusts, for exemple allergen solution (15), grain dust (10) and swine dust (11).

A major target of inhaled endotoxins is the alveolar macrophage. The number of alveolar macrophages increased in the study by Larsson et al (11) after exposure of dust containing endotoxin but not after pure endotoxin inhalation (5,14). Human alveolar macrophages are activated in vitro by very small amounts of endotoxin (<1 ng/mL), releasing several cytokines (e.g., TNF, IL-1, IL-6) and metabolites of archidonic acid (e.g., LTB4), this activity being increased in presence of endotoxin-binding protein (LBP) and soluble CD14 receptor (16). The cytokines and mediators released

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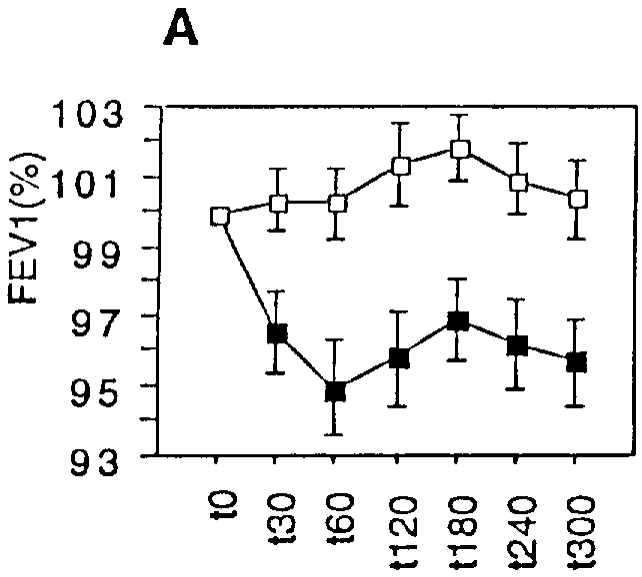
from the alveolar macrophages could stimulate the alveolar neutrophils and their recruitment into the lung while the release of TNF, IL-1 and IL-6 from the macrophages into the blood may induce the hepatic acute-phase protein response, like the C-Reactive Protein (CRP) and LBP. In both normal and asthmatic subjects, inhalation of pure endotoxin elicits a peak of CRP at 24 and 48 h (3,8), this response being dose-related (5). Six hours after an inhalation of endotoxin-contaminated dust, high concentrations of IL-1, IL-1 RA, IL-8 and TNF - and their specific mRNAs were measured in BAL while an increase in blood CRP was significant (10).

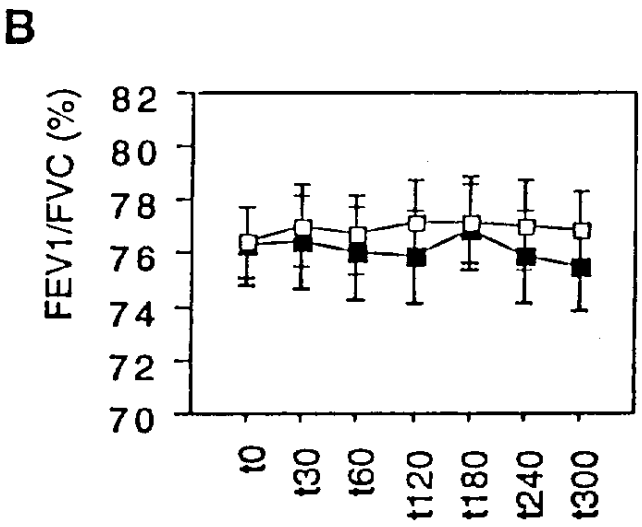
An alveolar (14) and sputum (5) rise in lymphocytes count was also observed after pure endotoxin inhalation. Exposure to dust contaminated by endotoxin was inconsistently associated with local infiltration by lymphocytes : an inhalation of grain dust containing endotoxin did not induce a change in BAL lymphocytes count (10) while swine dust induced both increase in the count and activation of BAL lymphocytes (17).

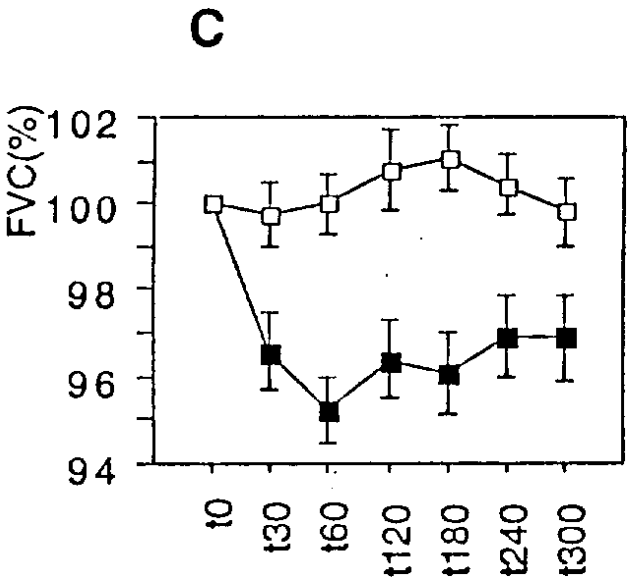
Several drugs have been evaluated for their blocking effects on the endotoxin response. The lung function response to inhaled endotoxin is blocked after pretreatment with sodium cromoglycate (18) and β_2-agonists, while an acute inhalation of cortocosteroid has no effect on the endotoxin induced blood inflammatory response.

Conclusion

Human bronchial challenges with endotoxin are useful for improving understanding of the physiopathology, defining threshold levels of acute response, determining dose-response relationships, studying possible individual sensitivities and evaluating the blocking effect of drugs on the response.







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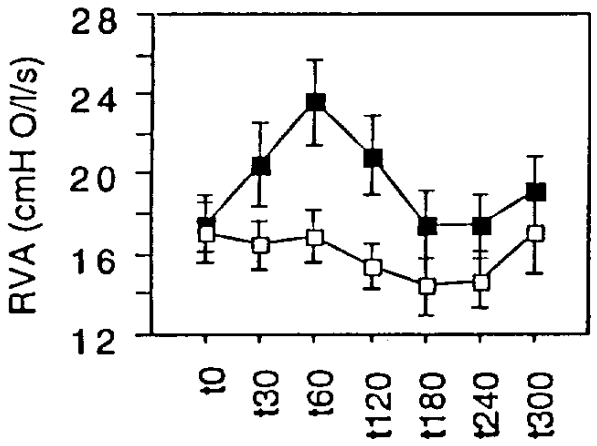

Figure 1. Lung function response after an inhalation of 20 µg endotoxin (■■) compared to the saline (□□) in 38 asthmatic subjects; FEV1, FVC, FEV/FVC are expressed in % (± SE) and RVA in cmH20/l/s (± SE).

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