Bronchial hyperreactivity is associated with enhanced grain dust-induced airflow obstruction

JOEL N. KLINE, PAUL J. JAGIELO, JANET L. WATT, AND DAVID A. SCHWARTZ
Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, Iowa 52242

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EPISTEMOLOGICAL STUDIES OF GRAIN WORKERS have demonstrated an excess of respiratory symptoms and airflow obstruction associated with chronic exposure to grain dust (6, 7, 12–14). Studies looking at specific host factors, such as atopy or the presence of specific antibodies to grain dust, have not found them to be consistently associated with either acute (11) or chronic (31) airway responses to grain dust. Other host factors, such as smoking, age, and duration of employment, have been associated with greater longitudinal declines in lung function (31). In addition, it appears that acute changes in airflow over a work shift or workweek are predictive of accelerated longitudinal declines in airflow (7, 20, 31). In fact, in grain workers with nonspecific bronchial hyperreactivity (BHR), there is an association between work-shift changes in forced expiratory volume in 1 s (FEV₁) and longitudinal declines in FEV₁, whereas no association was seen in workers with normal airway reactivity (15). These findings would suggest that BHR might be an important host factor contributing to the pathogenesis of chronic airflow limitation due to grain dust.

Airway inflammation appears to be essential to the development of grain dust-induced airflow obstruction. Our laboratory previously demonstrated that the endotoxin content of grain dust is an important determinant of the development (30) and progression (28) of airway disease among exposed workers and of the ability of grain dust to induce airflow obstruction and inflammatory responses in the airway (18, 19, 29). Inhaled endotoxin can induce airflow obstruction in naive or previously unexposed subjects, as well as those chronically exposed (9). Indeed, even among normal, nonatopic, nonasthmatic, nonsmoking subjects, some individuals exhibit a hypersensitive bronchospastic response after the inhalation of endotoxin (21).

The inflammatory response to inhaled grain dust is characterized by an exuberant chemotaxis of alveolar macrophages and neutrophils to the airways and alveolar spaces (8–10, 19, 34). Grain dust exhibits direct chemotactic activity for neutrophils (36) and induces the release of interleukin (IL)-1β (22) and other factors such as tumor necrosis factor-α (TNF-α), IL-6, and interleukin-8 were detected in bronchoalveolar lavage fluid 4 h after inhalation of CDE in all subjects, but no differences were detected between the control and BHR groups. These results suggest that, although subjects with BHR develop significantly greater percent declines in FEV₁ at time points up to 4 h after exposure to CDE. Significant increases in total cells, neutrophils, tumor necrosis factor-α, interleukin-6, and interleukin-8 were detected in bronchoalveolar lavage fluid 4 h after inhalation of CDE in all subjects, but no differences were detected between the control and BHR groups. These results suggest that, although subjects with BHR develop a more precipitous decline in FEV₁ after exposure to CDE, the inflammatory response to CDE in subjects with and without BHR.

inhalation exposure; airway inflammation; endotoxin; lipopolysaccharide

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multiple inflammatory mediators that are likely to be redundant and amplifying in effect.

The purpose of the present investigation is to further investigate the role of BHR as it relates to the acute physiological and inflammatory events due to acute grain dust inhalation. By using an acute-exposure model of grain dust-induced airway inflammation, our goal was to compare the acute physiological and inflammatory changes after exposure to grain dust in subjects with and without BHR. Our hypothesis was that both the physiological and inflammatory changes after exposure to grain dust were more pronounced in subjects with BHR and that airway inflammation would be associated with the development of airflow obstruction.

METHODS

We used a single-blind, crossover design in subjects with and without BHR to determine whether bronchial hyperreactivity (BHR) affected the acute physiological and inflammatory changes after acute inhalation of corn dust extract (CDE). All experimental protocols and consent forms were reviewed and approved by the Institutional Review Board (Human Subjects Review, Committee A) of the University of Iowa.

Study subjects. Subjects who were healthy, had never smoked, and were without any history of prior cardiac disease or occupational exposure to grain dust were recruited. Advertising requested nonsmoking subjects with no known lung disease or subjects with occasional respiratory symptoms. To be considered eligible for participation, all study subjects were required to have a normal physical examination, 12-lead electrocardiogram, chest X-ray, and pulmonary function tests (spirometry, lung volumes, diffusing capacity, and arterial blood gases). A standard histamine challenge test was performed on each subject, which included five inhalations of 0.03, 0.06, 0.12, 0.25, 0.5, 1.0, 2.5, 5.0, and 10.0 mg/ml concentrations of buffered histamine at room temperature, delivered according to the guidelines established by the American Academy of Allergy, Committee on Standardization of Bronchoprovocation (4). The cumulative dose (in breath units) of histamine causing a 20% fall in baseline FEV1 compared with diluent (sterile isotonic saline solution) or up to a maximum dose of 97.3 breath units was determined. Bronchial hyperreactivity was defined as a 20% or greater decrease in FEV1 compared with diluent FEV1, with a cumulative dose of histamine ≤47.3 breath units. The slope of the dose-response curve was calculated by dividing the maximal percent drop in FEV1 by the cumulative breath units causing this decline (27). Individuals in the study who were screened and found to have BHR were limited to subjects who were never previously diagnosed with asthma or who had a history of stable, mild, intermittent asthma with only occasional (less than twice per week) use of inhaled β-agonists. Subjects who were taking antihistamines, theophylline, inhaled corticosteroids, or other chronic medications were excluded from participation. All subjects were screened for atopy by using a standard panel of Aeroallergens and were nonatopic. Subjects on inhaled β-agonists were instructed to discontinue the drug for 24 h before both the histamine challenge and each inhalation exposure. Subjects with BHR were matched with subjects demonstrating normal airway reactivity and of similar age (within 5 yr), gender, and body height (within 5 cm) and weight (within 5 kg).

Protocol. All study subjects underwent two separate inhalation challenges (saline and CDE), with exposures separated by at least 2 wk. Previously, our laboratory demonstrated that lung function and lavage parameters return to baseline values within 48–96 h after inhalation of grain dust (10). To ensure continued participation in this trial, all subjects were exposed to saline on the first visit and CDE on the second visit, although the subjects were not informed about the order of the exposures. Vital signs, pulmonary function, and symptomatology were recorded before and after each inhalation exposure by using an established protocol.

Preparation of the CDE. Corn dust used in this study was obtained from the air-filtration system at an eastern Iowa grain facility. CDE was prepared by mixing 3.0 g of dust in 30 ml of sterile, pyrogen-free Hanks’ balanced saline solution (HBSS) without calcium or magnesium (0.1% solution), vortexing for 2 min, and shaking for 1 h at 4°C. The mixture was centrifuged at 800 g for 20 min, and the supernatant solution was collected, resulting in the CDE. The CDE solution underwent filter sterilization through a 0.22-µm filter (Acrocap Low Protein Binding Filter, Gelman Sciences, Ann Arbor, MI). All solutions used for inhalation were derived from a stock solution that underwent sterile filtration (bacteria and fungi) and endotoxin assay before separation into individual aliquots. These aliquots were stored at -70°C before use. Although levels of mycotoxins, such as aflatoxin and fumonisin, were not measured in these aliquots, only negligible concentrations have been previously detected in similar samples. Endotoxin concentration was measured by the endpoint chromogenic Limulus ameboocyte lysate assay (QCL-1000, Whittaker Bioproducts, Walkersville, MD). The measured endotoxin concentration in the CDE prepared by this method was 4.0 µg/ml.

Inhalation challenge. The solutions were administered via a nebulizer (model 646, DeVilbiss, Somerset, PA) and dosimeter (DeVilbiss), operated at 20 psi air pressure. Subjects, who were in the seated position during exposure and subsequent pulmonary function testing, controlled the timing of each nebulized dose and were instructed to inhale through the mouthpiece of the nebulizer and exhale through their nose. By using this delivery system and technique, a precise dose of inhalant was delivered. For each exposure, the goal was to administer 0.04 ml of inhalant (CDE or HBSS) per kilogram of body weight [or 0.16 µg lipopolysaccharide (LPS)/kg] by using continuous tidal respirations over a 60-min period of time. This dose of LPS was previously identified as equivalent to an average work-day inhalation exposure to LPS for a grain elevator worker (8, 9). Three of seven of the CDE inhalational challenges (but none of the saline exposures) to BHR subjects were terminated as a result of complaints by the subjects of severe chest tightness, dyspnea, or cough. Matched control subjects without BHR were then given equal amounts of CDE as the BHR subjects.

Pulmonary function testing. The pulmonary function tests consisted of serial measurements of airflow by a spirometer (Spirotech S-600, Graseby Anderson, Atlanta, GA). These maneuvers were performed by using standard protocols and American Thoracic Society guidelines (2). The spirometer was calibrated before each visit. With the subjects wearing nose clips and in a sitting position, spirometry was performed preexposure and at the following time points postexposure: 10, 20, and 30 min, and 1, 2, 3, 4, and 24 h.

Bronchoscopy. Bronchoscopy was performed 4 h after each inhalation exposure, in accordance with the standards established by the American Thoracic Society for bronchoscopy in asthmatic subjects (3). This time point was chosen because of previous studies in which airway inflammatory responses were assessed by bronchoscopy after exposure to grain dust extracts (8, 10). Subjects were pretreated with atropine in-
A total of 14 subjects participated in and completed the study (12 women, 2 men). The BHR subjects were matched by gender, age, weight, and height to control subjects without reactive airways (Table 1). As expected, there was a significant difference in both the slope of the dose-response curve to histamine between the control and BHR groups (Table 1) and the baseline FEV₁-to-forced vital capacity ratio (FEV₁/FVC) (Table 2) but not in any other measured pulmonary function parameter (Table 2).

Of the seven subjects with BHR, only four subjects were able to inhale the full intended dose (0.16 μg/kg endotoxin) of CDE. One subject developed bronchospasm after exposure to less than one-half of the calculated dose of CDE. A second subject with BHR received 33%, and another subject with BHR received 90% of the calculated dose, at which time they were unwilling to complete the exposure because of intolerable symptoms of chest tightness. The matched control subjects were given equal doses of CDE as the proband subjects with BHR. Although these control subjects without BHR received equivalent doses of CDE as the BHR subjects, none complained of symptoms requiring cessation of the protocol.

**Symptomatic response to inhaled CDE.** Respiratory and nonrespiratory symptoms were reported by subjects after exposure to CDE, including chest tightness, dyspnea, cough, sputum production, malaise, and chills. None of these symptoms was reported after inhalation of HBSS. When the frequency of these symptoms was compared in subjects with and without BHR, only chest tightness and dyspnea were found to be significantly different between these groups (Table 3). In subjects with BHR, chest tightness was experienced by a majority of the participants for at least the first 2 h postexposure, with subsequent decline. Only one control subject experienced chest tightness lasting more than 10 min. Similarly, four subjects with BHR experienced dyspnea lasting at least 1 h after inhalation of CDE, whereas no control subjects complained of dyspnea. There were no significant differences in the number of subjects reporting cough, chills, sputum

### Table 1. Demographics

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>BHR Subjects</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>26.6 ± 2.7</td>
<td>24.8 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Gender, female/male</td>
<td>6/1</td>
<td>6/1</td>
<td>NS</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>72.4 ± 6.4</td>
<td>74.4 ± 5.7</td>
<td>NS</td>
</tr>
<tr>
<td>Height, cm</td>
<td>167.5 ± 2.6</td>
<td>169 ± 4.2</td>
<td>NS</td>
</tr>
<tr>
<td>Histamine slope</td>
<td>0.05 ± 0.017</td>
<td>5.15 ± 2.39</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

Values are means ± SD. BH, bronchial hyperreactivity; NS, not significant. *Slope of the histamine dose-response curve [% change in forced expiratory volume in 1 s (FEV₁)/breath units histamine].

### Table 2. Baseline pulmonary function

<table>
<thead>
<tr>
<th>Function</th>
<th>Control Subjects</th>
<th>BHR Subjects</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁, liters (1 s)</td>
<td>3.28 ± 0.14(97)</td>
<td>3.21 ± 0.28(93)</td>
<td>NS</td>
</tr>
<tr>
<td>FVC, liters</td>
<td>4.02 ± 0.17(94)</td>
<td>4.43 ± 0.51(100)</td>
<td>NS</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>0.83 ± 0.09</td>
<td>0.74 ± 0.06</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SVC, liters</td>
<td>4.05 ± 0.18(95)</td>
<td>4.59 ± 0.60(105)</td>
<td>NS</td>
</tr>
<tr>
<td>RV, liters</td>
<td>1.55 ± 0.13(91)</td>
<td>1.44 ± 0.22(65)</td>
<td>NS</td>
</tr>
<tr>
<td>TLC, liters</td>
<td>5.61 ± 0.24(98)</td>
<td>6.03 ± 0.65(104)</td>
<td>NS</td>
</tr>
<tr>
<td>DlCO, ml CO·min⁻¹·mmHg⁻¹</td>
<td>31.8 ± 2.76(137)</td>
<td>31.6 ± 4.10</td>
<td>NS 31.7(134)</td>
</tr>
</tbody>
</table>

Values are means ± SD with % predicted in parentheses. FVC, forced vital capacity; SVC, slow vital capacity; RV, residual volume; TLC, total lung capacity; DlCO, diffusion capacity for carbon monoxide.
production, or malaise at each of the time points queried (data not presented).

**Pulmonary physiological response to inhaled CDE.** Acute airflow obstruction developed after exposure to CDE (but not after exposure to HBSS) in subjects both with and without BHR, occurring as early as 10 min postexposure and persisting for at least 4 h postexposure. This was demonstrated by declines in FEV₁ (Fig. 1) and in FEV₁/FVC (data not shown). Although both groups developed abrupt declines in FEV₁ within 10 min after inhalation of CDE, the BHR group had significantly greater declines in both FEV₁ and FEV₁/FVC. At 10 min postexposure, the mean percent decline in FEV₁ from baseline in subjects with BHR was 42%, which was significantly greater than control subjects (11%; \( P < 0.01 \)). Over the first 2 h after exposure to CDE, subjects with BHR continued to have significantly greater declines in FEV₁ compared with subjects with normal airway reactivity, although the magnitude of difference declined over time as a result of gradual improvement in FEV₁ in the BHR subjects. Interestingly, the greater percent decline in FEV₁ seen in the BHR group was associated with increased subjective reporting of chest tightness and dyspnea (Table 3).

**Inflammatory response to inhaled CDE.** An acute inflammatory response in the lower respiratory tract was observed after exposure to CDE compared with saline for normal control subjects as well as those with BHR (Fig. 2). The inflammatory response consisted predominately of increases in concentrations of total cells and neutrophils. Although these BAL cell concentrations increased significantly after inhalation challenge with CDE in both normal subjects and those with BHR, no differences were seen between these groups (Fig. 2).

In subjects with and without BHR, exposure to CDE (in comparison to saline) resulted in significant increases in the concentration of BAL fluid TNF-α, IL-6, and IL-8 (Fig. 3). However, post-CDE concentrations of TNF-α, IL-6, and IL-8 did not significantly differ between subjects with and without BHR.

**DISCUSSION**

Our results indicate that subjects with BHR develop greater respiratory symptoms and airflow obstruction after inhalation of CDE compared with subjects with no evidence of airway hyperreactivity. The initial marked decline in airflow obstruction appears to slowly...
LPS induced a small reduction in FEV$_1$ in asthmatic
enoic acid, PGE$_2$, or leukotriene B$_4$ in BAL fluid of
detectable levels of histamine, 15-hydroxyeicosatetra-
ously, our laboratory was not able to demonstrate
as through cholinergic pathways or nonadrenergic,
striction through neurally mediated mechanisms, such
rapid, short-term declines in airflow that may be exag-
in bronchoconstriction. These substances may cause
individuals with airway hyperreactivity
cause the release of histamine and leukotrienes from
interest. Extracts of grain dust have been shown to
exaggerated physiological response in subjects with
asthmatic individuals to inhaled endotoxin (23–25).
Michel and colleagues found that inhalation of 22 µg of
induced a small reduction in FEV$_1$ in asthmatic
but not in normal individuals (23) that was associated
increased nonspecific BHR (25). Our present
study bolsters these studies by demonstrating a signifi-
cantly greater degree of airflow obstruction after in-
halaion of CDE by subjects with BHR than was seen
in normal control subjects. These data support the
proposal that asthmatic individuals and those with
BHR are more likely to develop symptomatic airflow
obstruction when exposed to dusts containing high
levels of endotoxin. These findings may explain why
individuals with BHR develop more progressive airway
disease when working with grain dust (7).

The mechanism by which CDE produces an initial
exaggerated physiological response in subjects with
BHR was not explored in this study, but it is clearly of
interest. Extracts of grain dust have been shown to
cause the release of histamine and leukotrienes from
human lung tissue (5). Similarly, endotoxin, a major
component of grain dust, may cause the release of
preformed mediators such as histamine (32), resulting
in bronchoconstriction. These substances may cause
rapid, short-term declines in airflow that may be exag-
ergated in subjects with underlying BHR. Alterna-
tively, inhalation of CDE may cause acute bronchocon-
striction through neurally mediated mechanisms, such
as through cholinergic pathways or nonadrenergic,
noncholinergic neuropeptide mediators. However, pre-
viously, our laboratory was not able to demonstrate
detectable levels of histamine, 15-hydroxyeicosatetra-
enic acid, PGE$_{2\alpha}$, or leukotriene B$_3$ in BAL fluid of
normal control subjects 4 h after exposure to CDE (8).

More surprising than our finding of increased induc-
tion of airflow obstruction in subjects with BHR was
that the pulmonary inflammatory responses were not
different between subjects with and without BHR. In
an earlier study, Michel et al. (24) found a small but
significant increase in the concentration of plasma
TNF-α, peripheral leukocytosis, and neutrophils
among asthmatic subjects after inhalation of LPS. This
present study differs from previous studies in that the
protocol (delivered as CDE) resulted in delivery of a
significantly lower amount of inhaled endotoxin to the
subjects. The subjects were then evaluated by bron-
choscopy, a more specific measure of the airflow inflam-
matory response than measures of blood parameters.
Although both normal subjects and those with BHR
developed substantial airway inflammation after inha-
lation of CDE, there were no significant differences in
these inflammatory responses between the two groups.
There are a number of potential explanations for the
similar levels of inflammatory cells and mediators in
the BAL fluid obtained from the two groups after CDE
exposure. First, the lavage concentrations of cells and
cytokines are relatively crude indicators of airway in-
flammation in the region most pertinent to asthma.
Indeed, the BAL sample is more representative of
distal alveolar processes than the more proximal small
airways. Second, the cellular and protein mediators of
inflammation that we chose to measure, on the basis of
previous studies demonstrating their induction by en-
dotoxin and by grain dust (8–10), may not be the
mediators most relevant to the expression of broncho-
spasm. Alternative mediators may include neuropep-
dides, such as substance P, that are induced in a ham-
ster model by grain dust (16, 17) and blocked by the
anti-inflammatory agent dexamethasone (1). A poten-
tially more provocative explanation for the lack of
difference in induction of inflammation in subjects with
and without BHR is that airflow obstruction may ac-
tually provide protection from environmental stimuli.
Although we did not measure FEV$_1$ throughout the
exposure period, it is likely that reductions in FEV$_1$
were occurring during the period of inhalation chal-
lenge, as shown in nonasthmatic individuals in previ-
ous studies (21). This decrease in airflow may have
altered the distribution of aerosol in the lung, prevent-
ing aerosol from being deposited in the distal regions
of the lung in subjects with BHR. Thus BHR may act to
protect individuals from environmental exposures,
such as grain dust, by reducing the overall exposure,
resulting in less inflammation in the lower respira-
tory tract. In contrast, subjects with nonreactive airways
may be more likely to tolerate these exposures for
longer periods of time, but, as a consequence, they
develop greater airway inflammation in the lower re-
spiratory tract. Finally, the genetics of BHR (in this
study, as defined by sensitivity to inhaled histamine)
may differ from the genetics of the inflammatory re-
ponse to inhaled endotoxin. Our laboratory recently
demonstrated that both the inflammatory response
and the bronchospastic response to inhaled endotoxin
vary widely in normal, nonasthmatic subjects (21). The
inflammatory response to inhaled endotoxin may be
unrelated to BHR.

In conclusion, it appears that BHR is a major host
factor that is associated with exaggerated initial de-
clines in airflow after acute grain dust exposure but
may be protective in reducing the magnitude of the
acute inflammatory cell recruitment in the lower respi-
ratory tract. It is possible that the mechanism underly-
ing BHR, in conjunction with repetitive bouts of
bronchoconstriction and airway inflammation associ-
ated with chronic exposure to grain dust, may be
responsible for producing the chronic, irreversible airflow
obstruction. The significantly greater and more persis-
tent bronchospasm that follows inhalation of endotoxin-containing CDE by asthmatics may be responsible for the “healthy worker effect,” in which disease-susceptible individuals leave the work force. An important future study suggested by these findings includes comparison of the bronchospastic and inflammatory responses of workers occupationally exposed to grain dust who do or do not develop significant symptomatology. These results suggest that differences in symptoms and in the development of bronchospasm may not be reflected in different levels of airway inflammation between those groups.

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