The Effects of Inhalation of Grain Dust Extract and Endotoxin on Upper and Lower Airways*

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To characterize the short-term effects of grain dusts on pulmonary function, mucosal inflammation, and systemic responses, four women and three men inhaled nebulized corn and soybean dust extracts, endotoxin diluted with Hanks' balanced salt solution (HBSS), and HBSS. Subjects were volunteers recruited via newspaper advertisement and were required to be healthy, nonasthmatic, nonatopic never-smokers. The mean age was 26.9 years (range, 19 to 36 years). Using a randomized, double-blind, crossover design, each subject was challenged with each of the four substances with at least 10 days between challenges. Serial spirometry, peripheral blood leukocyte and differential cell counts, and 24-h postchallenge nasal lavages were performed. Extracts were produced by mixing 3 g of the corn or soybean dust with 30 ml HBSS followed by shaking for 60 min, centrifugation, then filter sterilization. The endotoxin solution was produced by mixing lyophilized Escherichia coli endotoxin (serotype 0111:B4) with HBSS to attain a final concentration of 7 mg/l, which was the same as the concentration of endotoxin in both dust solutions. The pH of all solutions and unmixed HBSS was adjusted to 5.8, which was the native pH of the soybean dust extract. Subjects were challenged with 0.08 ml/kg of each substance, resulting in a range of endotoxin doses of 30 to 60 μg, similar to that which a worker might inhale over the course of one workshift. The lowest mean percentage baseline FEV1 (±SD) after inhalation challenge was 99.2±2.1 for HBSS, and it was significantly lower for endotoxin (90.1±8.5, p = 0.03), corn dust extract (93.1±4.3, p = 0.02), and soybean dust extract (96.2±3.7, p = 0.03). In addition, a peripheral blood leukocytosis developed after exposure to all three endotoxin-containing solutions (p<0.05), yet a lower peripheral blood lymphocyte count was found only after inhalation of corn dust extract (p = 0.02). Interestingly, this was associated with a higher nasal lavage lymphocyte count after inhalation of corn dust extract (p = 0.03). Neither the decrease in peripheral blood lymphocytes nor the increase in nasal lymphocytes were found after inhalation of soybean dust extract or endotoxin. Our results indicate that extracts of grain dusts have physiologic effects similar to endotoxin. However, in spite of the same endotoxin levels, the effects of corn dust extract appear to have different biologic activity than either soybean dust extract or endotoxin.

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Grain handlers have been shown to have accelerated deterioration of pulmonary function when compared with civic workers.1-3 Reductions in airflow have been shown to occur over the course of one workshift and one work week.1,3,4,5 Both acute deterioration in lung function at work and increased airway reactivity, as assessed by methacholine challenge,6,7 appear to correlate with progressive loss of lung function in grain handlers.1,6-8

Inhalation of grain dust appears to induce an acute local inflammatory response as well as a systemic response. Increases in peripheral blood granulocyte counts occur between 2 and 24 h after inhalation of grain dust and aqueous grain dust extracts.6-9 Bronchoalveolar lavage (BAL) performed on workers exposed to grain dust reveals excess neutrophils in the initial portion of the lavage fluid.10 von Essen et al12 found that neutrophils had infiltrated the mucosa of the lower airways of guinea pigs 24 h after instillation of grain sorghum dust extract. Composite samples from the initial portion of BAL and nasal lavage obtained 24 h after inhalation of nebulized grain dust extract by human volunteers have also demonstrated a predominance of neutrophils.9,11

Recent investigation of the pathogens in grain dust has focused on the hypothesis that endotoxin, which contaminates all organic dusts, may be important in the initial inflammatory response.12,13 Dose-response relationships have been shown to exist between levels of respirable dust and symptoms of chest tightness and airflow obstruction in grain, cotton, and swine confinement workers.6,12-15 Spirometric abnormalities were more strongly associated with airborne endotoxin levels than with inspired dust concentrations in a...
Table 1—Order of Administration of Extracts*

<table>
<thead>
<tr>
<th>Inhaled Substance</th>
<th>HBSS</th>
<th>Endotoxin</th>
<th>Corn</th>
<th>Soy</th>
</tr>
</thead>
<tbody>
<tr>
<td>% first</td>
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<td>29</td>
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<td>14</td>
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<td>% third</td>
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<td>% fourth</td>
<td>14</td>
<td>43</td>
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</tr>
</tbody>
</table>

*Endotoxin = E. coli (serotype 0111:B4) endotoxin diluted in HBSS; Corn = aqueous extract of settled corn dust; Soy = aqueous extract of soybean dust.

recent study of animal feed workers.18 Furthermore, following exposure of healthy adults to Enterobacter agglomerans endotoxin, a dose-response relationship was demonstrated between the quantity of inhaled endotoxin and the severity of airflow obstruction. However, the dose of inhaled endotoxin used (200 μg) to achieve a significant response (FEV1, drop of 8.3 percent from baseline) was very high in comparison to most workplace exposures, and resulted in profound adverse systemic symptomatology.17

The purpose of this investigation was to compare the effects of inhaled nebulized corn and soybean dust extracts and endotoxin on airway function and inflammation. Our hypothesis was that the physiologic and biologic response to grain dust extracts and endotoxin would be similar as assessed by measures of airflow and mucosal cellularity. Our results indicate that inhalation of grain dust extracts and endotoxin results in airflow obstruction and peripheral blood leukocytosis. Interestingly, only the corn dust extract was associated with a drop in peripheral blood lymphocyte count and an increase in the concentration of nasal lavage lymphocytes.

METHODS

We used a double-blind, randomized, placebo-controlled, crossover design to evaluate the systemic, pulmonary, and mucosal inflammatory effects of inhaled nebulized endotoxin, corn dust extract, soybean dust extract, and pyrogen-free, calcium-free, and magnesium-free Hanks' balanced salt solution (HBSS) (Cell-Gro; Media-Tech, Inc., Herndon, Va).

Study Subjects

Subjects were required to be healthy never-smokers taking no prescription medicines, with no history of agricultural work. While no bronchoprovocative testing or skin testing was done, they were required to have no history consistent with asthma and no history suggestive of atopy (seasonal allergies or rhinitis). Subjects were recruited by a newspaper advertisement. Of 16 individuals volunteering for this investigation, 3 men and 4 women met the inclusion criteria and were selected as participants in the study. The mean age of the subjects was 26.9 years (range, 19 to 36 years).

Protocol

Subjects were exposed to each of four solutions (HBSS, endotoxin, corn, and soybean dust extracts) via inhalation challenge. Although the order in which the solutions were administered was randomly assigned, corn dust extract was the first or second inhaled solution for all subjects, and endotoxin was the third or fourth substance inhaled in six of the seven subjects (Table 1). Vital signs (temperature, BP, respiratory rate, and heart rate) and spirometry were measured before inhalation challenge, 30 min after, 1 h after, hourly until 8 h after challenge, and then again at 24 h. Peripheral blood leukocyte and differential cell counts were obtained prior to inhalation challenge and 6 h later. Nasal lavage was performed 24 h after inhalation challenge. Each inhalation challenge was separated by at least 10 days.

Preparation of Inhaled Solutions

Soybean dust was collected from an air filtration system in a soybean processing plant. Settled corn dust was collected from a grain (corn) elevator. While the sites at which the dusts were collected processed their respective grains exclusively, the degree to which these samples represent dust from similar grain facilities was not investigated. Extracts were produced by mixing 3 g of the dust with 30 ml HBSS followed by shaking for 60 min, centrifugation at 3,000 rpm, then filter sterilization of the supernatant using a 0.45 μm polyvinylidene difluoride (PVDF) (Acrocap; Gelman Sciences, Ann Arbor, Mich) filter.14 The endotoxin concentrations of both solutions were 7 mg/L as determined by the chromogenic Limulus amebocyte lysate assay (QCL-1000; Whittaker Bioproducts, Walkersville, Md). The pH of the corn dust extract was 5.3, and the pH of the soybean dust extract was 5.8. Lyophilized Escherichia coli endotoxin (serotype 0111:B4, Sigma Chemical Co, St. Louis, Mo) was diluted in HBSS to attain a final concentration of 7 mg/L. The pH of all solutions (including HBSS) was adjusted to 5.8. Sterility was confirmed by culture on tryptic soy agar at 30°C and 52°C, MacConkey's agar at 35°C, and malt extract agar at 25°C. All solutions were stored at −70°C until they were used.

Inhalation Challenge

The solutions were administered via a nebulizer (Devilbiss 646) and dosimeter (Devilbiss, Devilbiss Health Care Inc, Somerset, Pa), operated at 20 psi air pressure. The subjects controlled the timing of each nebulized dose and were instructed to inhale through the mouthpiece of the nebulizer and exhale through their nose. With this system and technique, we were able to maximize the amount of grain dust extract delivered to the mucosa of the airways. The mean extract dose delivered was 0.06 ml/kg. This resulted in delivery of between 4.5 and 8.1 ml to each subject, corresponding to between 30 and 60 μg of endotoxin. This dose corresponds to the expected range of endotoxin inhaled during a workshift in farming environments.15,16

Pulmonary Function Testing

The pulmonary function tests consisted of serial spirometry using a spirometer (MedGraphics CPF-S, MedGraphics Corporation, St. Paul, Minn). These maneuvers were performed using standard protocols and the American Thoracic Society guidelines.16

Nasal Lavage

Nasal lavage was performed 24 h after inhalation challenge. The subject tilted his head back, held his breath, and a 5-ml aliquot of sterile saline solution was instilled in one nostril, held for 10 s, then forcibly expelled. This was performed three times in each nostril. The lavage fluid was filtered through two layers of gauze, and it was centrifuged twice with resuspension of the cell pellet in 1 ml of HBSS. Cell counts were determined (Coulter Counter, Coulter Electronics Inc, Hialeah, Fla), and differential cell counts were performed using cytopsin preparation and staining (Diff-Quick, Baxter Scientific Products, Miami, Fla).

Statistics

The crossover design of this study dictated the analysis. Paired comparisons of spirometric data, vital signs, and peripheral blood
leukocyte and differential cell counts, and nasal lavage cell and differential cell counts were made between each solution type at each time point. Comparisons were also made to baseline data for peripheral blood leukocyte and differential cell counts for each grain type. The Wilcoxon Signed Rank Test was used for all analyses.

RESULTS

Percentage of baseline temperature was elevated significantly 3 and 4 h after inhalation challenge with the endotoxin solution when compared with inhalation challenge with HBSS. While the response after inhalation of the corn dust extract was similar to that of the endotoxin solution, it was not statistically different from HBSS. In contrast, the temperature response after inhalation of soybean dust extract was quite similar to that of HBSS. The heart rate mirrored the temperature responses, with the responses to both corn dust extract and endotoxin being significantly greater than to HBSS. Again, the response to soybean dust extract was most similar to that of HBSS. Respiratory rate was significantly greater 5 and 6 h after challenge with corn dust extract than after challenge with HBSS. However, inhalation of both endotoxin and soybean dust extract resulted in changes in the respiratory rate similar to those occurring after inhalation challenge with HBSS (vital signs data not shown).

Prechallenge spirometric parameters for individuals did not change significantly between challenges. All subjects developed airflow limitation after challenge with the endotoxin solution, corn, and soybean dust extracts. The baseline FEV1 after subjects inhaled the endotoxin solution and solutions prepared from corn and soybean dust extracts were, for most time points, significantly lower between 0.5 and 7 h postexposure than the FEV1 after inhalation challenge with HBSS (Fig 1, top). The maximal mean percentage of decrease in FEV1 ± SD after challenge with HBSS was 0.8 ± 2.1 (range, 0 to 3.6), after the endotoxin solution, 9.9 ± 8.5 (range, 1.0 to 22.0), after corn dust extract, 6.9 ± 4.3 (range 1.8 to 13.4), and after soybean dust extract, 5.8 ± 3.7 (range 0 to 10.12). For all inhaled substances, the maximal mean percentage of decrease from baseline occurred 30 min after challenge. At 8 and 24 h, these differences were still present; however, they were not statistically significant. The percentage of baseline FVC level was significantly lower after inhalation of corn dust extract than after HBSS 2 h postchallenge and at all subsequent time points, including 24 h postchallenge (Fig 1, center). This significantly lower baseline FVC was also seen between 1 and 5 h after challenge with endotoxin. After challenge with soybean dust extract, the percentage of baseline FVC was very similar to HBSS, but then gradually decreased, and became significantly different from HBSS 24 h postinhalation challenge. Thirty minutes after inhalation challenge, the percentage of baseline FEV1/FVC level measured for endotoxin, corn, and soybean dust extracts decreased significantly compared with HBSS (Fig 1, bottom). Thereafter, with the exception of several isolated time points, these ratios, though consistently lower, were not significantly different from those after challenge with HBSS.

Baseline peripheral blood leukocyte counts were not significantly different between subjects prior to the inhalation challenges. Total peripheral blood leukocyte counts 6 h after inhalation of endotoxin and extracts prepared from corn and soybean dusts were significantly higher than their respective baseline values (data not shown), and in comparison to the
response following inhalation of HBSS (Fig 2). These relationships were also observed for neutrophil counts; however, there were no increases in immature forms or eosinophils. Interestingly, absolute peripheral blood lymphocyte counts were significantly lower 6 h after inhalation challenge with corn dust extract than after HBSS (Fig 3, p = 0.03). Although a decrease in the peripheral lymphocyte count was also seen after challenge with both endotoxin and soybean dust extract, these differences were not statistically significant.

Total and percent lymphocyte counts in nasal lavage fluids collected 24 h after challenge with corn dust extract were significantly greater than in specimens collected 24 h after challenge with HBSS (Fig 4, p = 0.03). While nasal lavage lymphocytes were also greater than HBSS 24 h after inhalation of the endotoxin solution and the soybean dust extract, these were not statistically different from the response to HBSS. There were no other significant differences observed in nasal lavage cellularity associated with the specific inhalation challenges.

**Discussion**

Our results indicate that normal subjects develop mild airflow limitation after inhalation of filter-sterilized grain dust extracts and that this decrease in airflow is similar in severity and duration to that which occurs after inhalation of endotoxin. This is accompanied by a peripheral blood leukocytosis with a predominance of granulocytes. Interestingly, inhalation challenge with corn dust extract was associated with a drop in peripheral blood lymphocytes and a higher concentration of lymphocytes in the nares than after inhalation of HBSS. Although there were differences in the responses to all of the inhaled substances, in general, settled corn dust extract, soybean dust extract, and endotoxin all resulted in a similar physiologic response when compared with HBSS. However, in spite of similar levels of endotoxin exposure, corn dust extract appears to have different biologic activity than either soybean dust extract or endotoxin alone.

The predominant physiologic effect following inhalation challenge with grain dust extracts and endotoxin was the development of airflow obstruction. This was most pronounced in the first 30 min, with subsequent improvement. The initial response to all substances was remarkably similar; however, the subsequent courses were somewhat different. Interestingly, the FVC steadily improved after inhalation of endotoxin, but it remained at a significantly lower level than after HBSS and corn dust extract, and actually slowly worsened after inhalation of soybean dust extract, finally becoming significantly lower than after HBSS at 24 h. The differences in the effects of these substances are consistent with the hypothesis that while their immediate effects are likely due to endo-

![Figure 2](image1.png)

**FIGURE 2.** Percent baseline peripheral blood WBC count 6 h after inhalation challenge. HBSS = Hanks' balanced salt solution; endotoxin = E. coli (serotype 0111:B4) endotoxin diluted in HBSS; corn = aqueous extract of settled corn dust; soy = aqueous extract of soybean dust. Asterisk = p<0.05 compared with HBSS (Wilcoxon).

![Figure 3](image2.png)

**FIGURE 3.** Percent baseline peripheral blood lymphocyte count 6 h after inhalation challenge. HBSS = Hanks' balanced salt solution; endotoxin = E. coli (serotype 0111:B4) endotoxin diluted in HBSS; corn = aqueous extract of settled corn dust; soy = aqueous extract of soybean dust. Asterisk = p<0.05 compared with HBSS (Wilcoxon).

![Figure 4](image3.png)

**FIGURE 4.** Nasal lavage lymphocyte counts 24 h after inhalation challenge. HBSS = Hanks' balanced salt solution; endotoxin = E. coli (serotype 0111:B4) endotoxin diluted in HBSS; corn = aqueous extract of settled corn dust; soy = aqueous extract of soybean dust. Asterisk = p<0.05 compared with HBSS (Wilcoxon).
toxin, there is an additional component responsible for delayed toxicity that was not present in the endotoxin solution.

This is further supported by the different peripheral blood leukocyte response observed after challenge with the extract prepared from corn dust compared with endotoxin and soybean dust extract. Interestingly, peripheral lymphocyte counts actually dropped after inhalation of corn dust extract and were significantly lower than the lymphocyte counts 6 h after inhalation of HBSS. Twenty-four hours after inhalation challenge, there were significantly more lymphocytes in the nasal lavage fluid after inhalation of corn dust extract than after inhalation of HBSS, suggesting recruitment of peripheral blood lymphocytes into the mucosa of the upper airways. These differences were present, but not statistically significant, after inhalation of endotoxin and soybean dust extract.

The most common organism found in vegetable dusts is *Enterobacter agglomerans*. The use of *E. coli* endotoxin in the endotoxin solution may contribute to these differences, but it is likely that there are components other than endotoxin that are important pathogenic constituents of grain dusts. Rask-Anderson and colleagues found no relationship between high levels of airborne endotoxin and symptoms in farmers. Buck and colleagues have shown that 63 to 70 percent of the bronchoconstricting activity remains after removing endotoxin when nebulized cotton bract extracts are inhaled. Von Essen et al have shown that grain dust extracts recruit neutrophils via alveolar macrophage activation and complement activation. Importantly, neutrophil chemotactic activity was preserved after endotoxin depletion. Tannins found in cotton bract extracts and in grain dust have been found to stimulate neutrophil migration, fix complement, and have significant effects on chloride secretion of airway epithelium. Mycotoxins are present in significant quantities and may contribute to the bioactivity of the extracts. Thus, although it is likely that endotoxin in agricultural dust is important in causing pulmonary dysfunction, its exact role is not clear, and it is not likely to be the only factor involved.

Although there are no other published direct comparisons between the effects of different types of grain dusts in humans, the literature suggests that different dusts have different effects. In our study, corn dust extract appeared to have the most biologic activity (as assessed by nasal lavage cellularity) of the three endotoxin-containing substances. Inhalation of both corn dust extract and endotoxin resulted in elevated body temperature compared with HBSS and soybean dust extract. There were also significantly higher heart and respiratory rates after inhalation of corn dust extract, in spite of the same endotoxin concentrations in the two extracts and the endotoxin preparation. Although crude, these parameters suggest a different metabolic response to the inhaled corn dust extract than to the soybean dust extract. The physiologic effects of soybean dust extract were less pronounced than those of the corn dust extract, and by several parameters, it was less biologically active than the endotoxin solution. As these subjects were nonsmokers, had never been exposed to soybean dust, and had no history of atopy, this supports the hypothesis that sensitization must occur to exhibit the acute, severe airflow obstruction that has been seen in Barcelona and other European cities.

These observations regarding the differences between the various endotoxin-containing substances in our study must be viewed with caution. Our randomization procedure resulted in all subjects receiving corn dust extract as one of the first two substances inhaled, and all but one of the subjects were exposed to the endotoxin solution as one of the last two substances. Thus, the most severe effects observed following inhalation of corn dust extract may have resulted from the sequence in which these solutions were inhaled. However, the order of administration does not appear to account for the differences we observed between the mild effects of soybean dust extract and the more severe effects of the endotoxin.

In this study, subjects inhaled doses of endotoxin comparable to those to which workers might be exposed during one workshift. Airborne endotoxin levels in the desirable fraction of dusts to which farmers are exposed have been measured at 2 μg/m³ to over 50 μg/m³. Assuming a worker inhales 15 m³ per 8 h workshift, he would inhale 30 to 750 μg endotoxin during a full day of work. In this study, subjects inhaled 30 to 60 μg endotoxin in HBSS, soybean dust extract, and corn dust extract.

The effects of inhalation of high concentrations of agricultural dusts have been well documented. They consist of flu-like symptoms with fever, leukocytosis, and airflow obstruction, and has been referred to as the organic dust toxic syndrome. It has been hypothesized that the responsible agent in these bioaerosols is endotoxin. Our results indicate that extracts of grain dust have physiologic effects similar to those of endotoxin; however, the systemic and biologic activity may be somewhat different from pure endotoxin and may be specific to the type of grain product. Further studies are needed to identify the specific components of grain dust that are responsible for these differences. In addition, the early biologic response to grain dust needs to be investigated further.

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