Grain Dust-induced Airflow Obstruction and Inflammation of the Lower Respiratory Tract

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To investigate the relationship between the physiologic and biologic effects of grain dust inhalation, we exposed 15 nonsmoking, nonasthmatic, nonatopic male grain handlers to buffered saline and aqueous corn dust extract by inhalation challenge in a crossover study. The inhalation challenges to buffered saline and corn dust extract were separated by at least 14 d. Compared with buffered saline, inhalation of corn dust extract resulted in significant airflow obstruction, which was observed within 30 min of exposure and persisted for 5 h. Inhalation of corn dust extract resulted in an acute inflammatory response characterized by higher concentrations of neutrophils ($p = 0.001$), IL-1β ($p = 0.001$), IL-1RA ($p = 0.001$), IL-6 ($p = 0.001$), IL-8 ($p = 0.001$), and TNF-α ($p = 0.04$) in bronchoalveolar lavage (BAL) fluid. mRNA levels specific for IL-1β, IL-1RA, IL-6, and IL-8 from cells present in the BAL fluid were significantly greater after challenge with corn dust extract than after challenge with buffered saline. Importantly, no significant differences were observed in the concentration of lymphocytes or eosinophils in the BAL fluid following inhalation of corn dust extract, and the concentrations of histamine and 15-HETE were similar in BAL fluid after the two challenges. The maximal percentage decrease in FEV_1 was significantly associated with the absolute neutrophil concentration in the BAL fluid ($p = 0.001$), as well as the concentration of TNF-α ($p = 0.03$), IL-1β ($p = 0.005$), IL-1RA ($p = 0.001$), IL-6 ($p = 0.001$), and IL-8 ($p = 0.001$) in the BAL fluid. These findings indicate that grain workers who inhale corn dust extract develop airflow obstruction in association with a brisk neutrophilic inflammatory response, as well as increased levels of proinflammatory cytokines in the lower respiratory tract. Furthermore, cytokine release is accompanied by upregulation of the corresponding mRNA. These physiologic and biologic responses to inhaled corn dust are not dependent on preexisting asthma or the presence of atopy, and our results suggest that classic immunological mechanisms do not initiate this inflammatory response. In fact, our results suggest that the airway response to grain dust represents an acute inflammatory response to an inhaled toxin, such as endotoxin. **Clapp WD, Becker S, Quay J, Watt JL, Thorne PS, Frees KL, Zhang X, Koren HS, Lux CR, Schwartz DA. Grain dust-induced airflow obstruction and inflammation of the lower respiratory tract. Am J Respir Crit Care Med 1994;150: 611-7.**

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Chronic inhalation of grain dust has been shown to adversely affect pulmonary health (1). Up to 5 million agricultural workers are exposed to grain dust each year in North America (1–3), and between 15 and 37% of nonsmoking grain handlers develop chronic bronchitis (4) and have impaired pulmonary function (5–7). Longitudinal studies have shown accelerated deterioration in pulmonary function in these grain workers (8), the severity of which appears to be related to the concentration of airborne grain dust in the work environment (7, 9). These levels of airborne grain dust are related to the presence of bronchial hyperreactivity, as well as to the development of airflow limitation across a work shift (10–13). Accordingly, both factors have been shown to predict accelerated deterioration in pulmonary function in these workers (8, 14). Thus, inhalation of grain dust has been shown to cause acute and chronic airway injury characterized by bronchitis and airflow obstruction. Moreover, the acute responses appear to be predictive of the long-term responses.

Airway inflammation is clearly an important characteristic of

asthma. Asthmatic subjects have been shown to have increased mast cells and eosinophils in bronchoalveolar lavage fluid and in the airway mucosa (15, 16). In addition to abnormally elevated levels of histamine and several eicosanoids (17), other inflammatory mediators, such as granulocyte-macrophage colony-stimulating factor, interleukin-6 (IL-6), and interleukin-8, are increased in the lower respiratory tracts of asthmatic subjects (18). Furthermore, bronchial hyperresponsiveness has been shown to be related to sloughing of airway epithelia (19, 20) and abnormal vascular permeability (21).

Inhalation of grain dust causes an inflammatory response characterized by neutrophil recruitment to the lower respiratory tract (22, 23). *In vitro* studies indicate that extracts of grain dust are capable of recruiting neutrophils by several mechanisms, including endotoxin- and nonendotoxin-induced chemotaxis, activation of complement, and release of alveolar macrophage-derived neutrophil chemotactic activity (22). Thus, although evidence exists for grain dust-induced neutrophilic inflammation in the lower respiratory tract of humans, further research is needed to characterize the important components of this inflammatory response and the relationship between the inflammatory response and the development of airflow obstruction.

The hypothesis of this study is that inhalation of grain dust results in an inflammatory response in the lower respiratory tract of grain handlers and that this inflammatory response involves the production of several important cytokines. Moreover, we hypothesize that this biologic response is associated with the development of airflow obstruction. To test these hypotheses, we used a crossover study designed to compare physiologic and biologic manifestations of the responses to inhaled buffered saline solution and an aqueous extract of corn dust in 15 grain workers.

METHODS

Selection of Subjects

Our study population was selected from approximately 200 grain handlers in eastern Iowa who are participating in a population-based longitudinal study of grain dust-induced lung disease. As part of this study, airway hyperresponsiveness was evaluated using an abbreviated histamine challenge (24, 25), and atopic status was determined using a standard battery of 10 aeroallergens common in eastern Iowa and seven airborne commercial allergens prepared from mixed grain, corn, and soybeans (DermaPik® system; Greer Laboratories, Lenoir, NC). Subjects selected for this investigation were required to be nonatopic and to have a negative histamine bronchoprovocation test (24, 25). Subjects were also required to have not smoked cigarettes for at least 2 yr, have less than a 10 pack-year smoking history, have no underlying medical illness, and have no current medication use. Each subject was also required to have an unremarkable chest radiograph and electrocardiogram. Of 43 grain handlers asked to participate, 25 refused and 18 consented to participate in all phases of this investigation; 15 completed the protocol, two elected to discontinue the experiment after the first exposure, and one was eliminated from the protocol before the first exposure because of clinically significant hypertension.

Protocol

All subjects were admitted to the Clinical Research Center at the University of Iowa Hospitals and Clinics the night before the inhalation challenge, and a standard protocol was followed in all cases. A screening evaluation, which included a complete history and physical examination, pulmonary function tests (spirometry, lung volumes, and diffusing capacity of carbon monoxide [D_{LCO}]), a chest X-ray, and an electrocardiogram, were performed on the evening of admission. At 6:30 the next morning, a plastic intravenous catheter was placed in the right forearm (heparin lock) and a peripheral blood sample was obtained. At 7:00 A.M., baseline forced expiratory maneuvers were performed using a Spirotech® S-500 spirometer (Graseby Anderson, Atlanta, GA) with standard protocols and following American Thoracic Society guidelines (26). Subjects were then exposed to nebulized buffered saline (first visit) or corn dust extract (second visit, at least 14 d following the first visit) by inhalation challenge. The aerosol challenge lasted approximately 60 min. Vital signs and forced expiratory maneuvers were measured at 30 min, 1 h, and hourly after the inhalation challenge was completed. Peripheral venous blood was obtained 5 h after inhalation challenge, and 1 h later, bronchoscopy was performed. Blood samples were processed for leukocyte and differential cell counts by our hospital clinical laboratories using standard protocols.

Inhalation Challenge

The solutions were administered via a DeVilbiss 646 nebulizer and DeVilbiss dosimeter (DeVilbiss Health Care Inc., Somerset, PA) operated at an air pressure of 20 psi. The subjects controlled the timing of each nebulized dose by arming the dosimeter before inhalation. The dosimeter automatically discharged for 0.6 s when triggered by the pressure drop in the nebulizer from inhalation. Thus, nebulization of the solution occurred only during inspiration. The port cap of the nebulizer was closed, and the subject exhaled through his nose. Therefore, the system was closed, restricting movement of the aerosol to the nebulizer, the subject's oral cavity and airway. The dose delivered was measured by changes in weight of the nebulizer. We filled the nebulizer with up to 2.5 ml solution, weighed the nebulizer, nebulized the solution using the dosimeter until the nebulizer was dry, and weighed the nebulizer again. This was repeated until the subject had inhaled 0.08 ml/kg. Because the nebulizer's reservoir was dry at the end of the challenge, we assume that a progressively concentrated solution would have been nebulized. Using this method, we were able to deliver doses to within 0.001 ml/kg of the target dose. This resulted in delivery of between 4.5 and 8.1 ml to each subject, corresponding to between 30 and 60 μg endotoxin, a dose that would be inhaled by an agricultural worker in a dusty environment over the period of an 8-h work shift (27, 28).

Preparation of Inhaled Solutions

Corn dust was obtained from a collection receptacle of an air filtration system at a corn storage facility. The dust in this receptacle had accumulated over the 2 wk before collection. The dust was placed in plastic containers, which were sealed and stored at 9° C. Extracts were produced by mixing 3 g dust with 30 ml buffered saline (Hanks' balanced salt solution; Media-Tech, Inc., Herndon, VA), followed by shaking for 60 min, centrifugation at 3,000 rpm, and then filter sterilization of the supernatant using a 0.45-μm polyvinylidine difluoride filter (Acrocap®; Gelman Sciences, Ann Arbor, MI) (22). The endotoxin concentrations of the corn dust extract solutions was 7 μg/ml as determined by the chromogenic *Limulus* amebocyte lysate assay (QCL-1000; Whittaker Bioproducts, Walkersville, MD). Sterility was confirmed by culture on trypticase soy agar at 35 and 52° C, McConkey's agar at 35° C, and malt extract agar at 25° C. The pH of the buffered saline was adjusted to 5.3, which was the measured pH of the corn dust extract. The solutions were stored in 15-ml aliquots at −70° C before use.

Bronchoscopy

Bronchoscopy was performed in accordance with the standards established by the American Thoracic Society for bronchoscopy in asthmatic subjects (29). Subjects were premedicated with 50 to 100 mg meperidine and 0.6 mg atropine intramuscularly. Lidocaine (4%) was aerosolized to anesthetize the larynx topically. Supplemental oxygen was administered, and the subjects were monitored with a single-lead cardiac monitor, automatic blood pressure cuff, and pulse oximeter. Immediately before beginning the procedure, midazolam was administered intravenously and titrated to the comfort of each subject.

Bronchoalveolar Lavage

An Olympus P-10 (1.5 mm channel; Olympus, Lombard, IL) bronchoscope was introduced through the oral cavity. The vocal cords, carina, and left mainstem bronchus were anesthetized with 3 ml of 2% lidocaine. The bronchoscope was gently passed through the trachea, introduced into

the right middle lobe, and wedged in the medial segment, where 20 ml of 37° C sterile 0.9% (normal) saline solution was introduced. Immediately afterward, suction was gently applied (60 mm Hg), and the effluent was collected in a 50-ml specimen trap (Cheesebrough-Ponds, Inc., Greenwich, CT). This was repeated five more times, for a total lavage volume of 120 ml. The return of the first 20-ml aliquot was kept separate from the other aliquots (which were combined), thus separating the "airway fraction" from the "alveolar fraction" of the bronchoalveolar lavage (BAL) fluid (30). At the second visit, the BAL was performed in a subsequent of the lingula.

Processing of Specimens

Immediately following the bronchoscopy, the BAL fluid was strained through two layers of surgical 4 × 4 gauze into 50-ml conical tubes. The volume was noted, and the tubes were centrifuged for 5 min at 200 x *g*. The supernatant fluids were decanted and frozen at −70° C for subsequent use. The residual pellet of cells was resuspended and washed twice in Ca^{2+}- and Mg^{2+}- free Hanks' balanced salt solution (Sigma Chemical Co., St. Louis, MO). After the second wash, 100 μl solution was removed for cell count using a hemacytometer (Reichert-Jong, Horsham, PA), and 10 μl was then removed for differential cell counts using a cytospin preparation and DiffQuik® staining (Baxter Scientific Products, Miami, FL). The cells were washed once more and resuspended at 1 × 10^6 cells/ml in RPMI 1640 medium; 1 × 10^6 cells were also suspended in 1 ml of 4 M guanidine isothiocyanate (GITC), and RNA was prepared from the cells by centrifugation of 0.1 ml of the GITC suspension over 0.1 ml cesium chloride by the miniprep method (31).

Measurement of Cytokines in BAL Fluid and Serum

Tumor necrosis factor α (TNF-α) bioactivity was tested with a cytotoxicity assay using the TNF-sensitive L929 mouse fibroblast cell assay (32). Interleukin-1β (IL-1β) was measured using a standard ELISA (R&D Systems, Minneapolis, MN) prepared with a rabbit monoclonal antibody against IL-1β. The antiserum used in this assay has been shown to be specific for IL-1β, with no cross-reactivity with IL-1α, interleukin-2, TNF-α, or interferon-γ. Interleukin-1 receptor antagonist (IL-1RA) was determined using a standard ELISA (R&D Systems, Minneapolis, MN) prepared with antibodies raised against recombinant human IL-1RA. This assay is highly specific and is sensitive to 22 pg/ml. Interleukin-6 (IL-6) was measured using a standard ELISA (R&D Systems, Minneapolis, MN) prepared with antibodies raised against recombinant human IL-6. This assay is highly specific, with no demonstrable cross-reactivity, and is sensitive to 0.35 pg/ml. Interleukin-8 (IL-8) was measured using a standard commercially available ELISA (R&D Systems, Minneapolis, MN) prepared with recombinant human antibodies raised against human IL-8. This assay is highly specific, with no demonstrable cross-reactivity, and is sensitive to 4.7 pg/ml. Histamine was measured using an ELISA prepared with high-affinity monoclonal antibodies directed against histamine (Immunotech International, Marseilles, France). The assay is sensitive to 0.2 nM. 15-Hydroxyeicosatetraenoic acid (15-HETE) was measured using a standard radioimmunoassay (RIA) (Advanced Magnetics, Inc., Cambridge, MA) and rabbit antihuman 15-HETE antibodies. Leukotriene B_4 (LTB_4) was measured using a standard RIA (Advanced Magnetics Inc., Cambridge, MA) and rabbit antihuman LTB_4 antibodies. Prostaglandin E_2 (PGE_2) was measured using a standard RIA (Advanced Magnetics, Inc., Cambridge, MA) and rabbit antihuman PGE_2 antibodies.

Semiquantitative PCR Technique for Determination of Relative Cytokine mRNA Levels

A semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR) was used to determine relative amounts of cytokine mRNA present in cells obtained by BAL. Samples of cells frozen at −70° C in GITC were thawed, and 0.1 ml GITC solution was layered on 0.1 ml of 5.7 M cesium chloride for isolation of RNA (31). RNA corresponding to 10^4 cell equivalents was reverse transcribed with 500 U Moloney murine leukemia virus RT (GIBCO/BRL, Gaithersburg, MD) in a buffer containing 10 mM TRIS HCl (pH 9.3), 50 mM KCl, 3 mM $MgCl_2$, 100 μg/ml bovine serum albumin (BSA), 50 U RNAsin (Promega, Madison, WI), 1 mM dNTP (Pharmacia, Pleasant Hill, CA), and 1 μM random hexamers (Pharmacia) at 39° C for 45 min. PCR was performed on 2 μl cDNA in a buffer consisting of 10 mM TRIS HCl, 3 mM $MgCl_2$, 100 μg/ml BSA, 2.5 mM dNTP, 1.25 U Taq polymerase, and 200 nM cytokine-specific primers. The actin primers corresponded to bp 835–856 (sense) and bp 1,015–1,035 (antisense) of the β-actin cDNA sequence as described by Ponte and colleagues (33). The primer pairs for TNF-α and IL-1β were synthesized according to sequences described by Wang and colleagues (34). The primer sequence synthesized for IL-1RA mRNA expression was the same as described by Haskill and colleagues (35). The primer pair for IL-6 was made to bp 42–61 (sense) and to bp 334–354 (antisense) of the IL-6 cDNA sequence (36); the IL-8 primer pair was made to the bp 147–174 (sense) and bp 324–266 (antisense) of the IL-8 cDNA sequence (37).

To determine relative cytokine mRNA in the BAL cell preparations, a standard curve (1:5 dilutions) of cDNA derived from alveolar macrophages stimulated with endotoxin *in vitro* (1 μg/ml for 6 h) was amplified in parallel with the unknown cDNAs. One mRNA unit corresponded to RT-PCR product derived from 1 ng macrophage RNA. cDNA was amplified in a 96-well thermocycler (M.J. Research, Watertown, MA), 94° C, 1 min; 56° C, 2 min; and 72° C, 2 min for 30 to 35 cycles. The PCR products were electrophoresed into 2% agarose gels (IBI, New Haven CT), which then were stained for 15 min with ethidium bromide (5 μg/ml) and photographed with Type 55 positive/negative film (Polaroid). The negatives were scanned for optical density using a Visage® image analyzer (Millipore, BioImage Products, Ann Arbor, MI), and sample mRNA units were derived from the macrophage standard curves run for the same number of cycles.

Statistics

The distribution of the data required that nonparametric tests be performed, and the crossover design of the study allowed paired analyses. Thus, the Wilcoxon signed-rank test was used to make comparisons between the data generated from each inhalation challenge (38). Spearman's rank correlation was used to examine the relationships between the changes in spirometric parameters and the markers of airway inflammation (38).

RESULTS

The mean age of the study subjects was 34.7 (range 18 to 56) yr. The study subjects had been employed a mean of 10.1 (range 1 to 25) yr in the grain-handling industry. Eight of the subjects worked exclusively with corn, and seven worked at sites that processed mixed grains. All subjects were never smokers, and as stipulated by our selection criteria, all subjects were nonatopic and had a negative airway response to inhaled histamine. Baseline measures of pulmonary function are shown in Table 1.

Inhalation challenge with buffered saline resulted in minimal increases in FEV_1, FVC, and the FEV_1/FVC ratio (Figure 1). However, within 30 min after the inhalation challenge with corn dust extract, statistically significant, clinically relevant declines in FEV_1, FVC, and FEV_1/FVC were observed. The obstructive physiology

TABLE 1

BASELINE SPIROMETRIC AND GAS-EXCHANGE PARAMETERS*

Parameter	Value	Range
FEV_1	91.5 ± 3.0	69.0–108.1
FVC	91.6 ± 2.7	68.1–110.2
FEF_{25-75}	77.0 ± 4.9	39.9–101.9
TLC	106.9 ± 3.3	80.4–124.7
RV	110.4 ± 8.7	55.6–172.2
FRC	91.1 ± 3.6	64.9–110.9
D_{LCO}	106.2 ± 3.5	79.5–129.1
FEV_1/FVC	77.5 ± 1.6	63.9–87.0
Pa_{O_2}	82.6 ± 2.9	60.6–105.0

Definition of abbreviations: FEF = forced expiratory flow; RV = residual volume; SEM = standard error of the mean.

* Pulmonary function values are expressed as the percentage predicted (mean ± SEM and range), except for the FEV_1/FVC ratio and the Pa_{O_2}, which are expressed as absolute values.

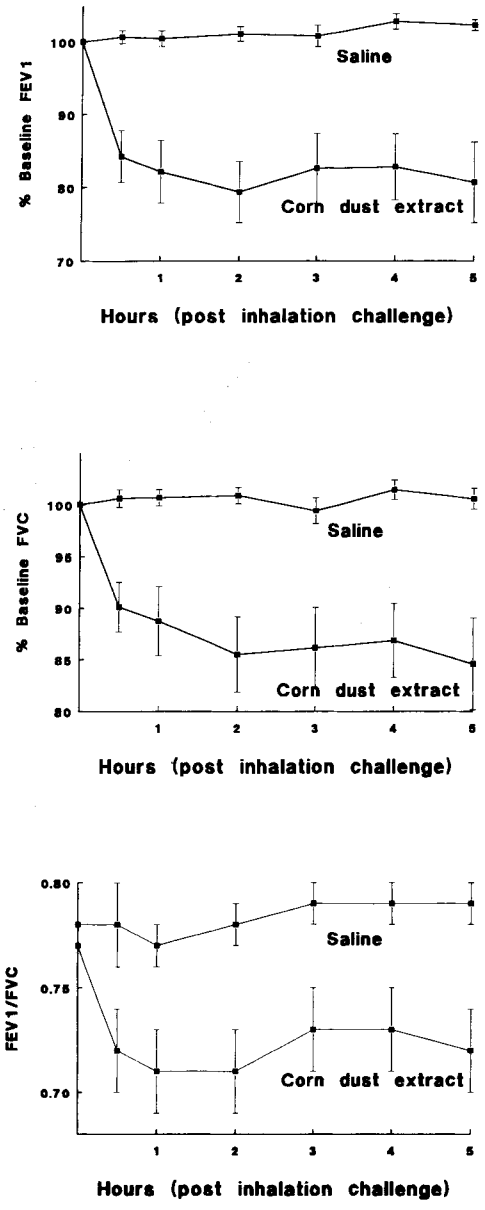

Figure 1. Percentage of baseline FEV_1 (*top*) and FVC (*middle*), and FEV_1/FVC (*bottom*) after inhalation challenges with buffered saline and corn dust extract (± SEM).

TABLE 2

VITAL SIGNS (OBTAINED AT REGULAR INTERVALS AFTER INHALATION CHALLENGE) AND PERIPHERAL VENOUS LEUKOCYTE COUNTS (MEAN ± SEM)

	Buffered Saline	Corn Dust Extract	p Value
Maximum temperature, °C	36.6 ± 0.2	37.5 ± 0.8	0.001
Maximum heart rate, beats/min	68.9 ± 9.9	83.7 ± 14.4	0.004
Maximum respiratory rate, breaths/min	18.3 ± 3.1	22.7 ± 4.3	0.009
Minimum systolic blood pressure, mm Hg	110.2 ± 6.5	112.4 ± 8.6	0.382
Minimum diastolic blood pressure, mm Hg	69.0 ± 9.5	65.3 ± 4.3	0.071
White blood cells 6 h after challenge, cells/mm^3	7,229 ± 586	15,001 ± 928	0.001
Neutrophils 6 h after challenge, cells/mm^3	4,880 ± 654	12,664 ± 918	0.001

associated with the inhalation of corn dust extract persisted for the subsequent 5 h until bronchoscopy was performed.

Inhalation of corn dust extract resulted in several constitutional changes. Maximal temperature, heart rate, and respiratory rate were all significantly higher after inhalation of corn dust extract than after buffered saline (Table 2). However, no significant effect on blood pressure was observed. Furthermore, peripheral blood leukocytes and neutrophils were significantly higher 5 h after inhalation challenge with corn dust extract. Importantly, serum concentrations of TNF-α and IL-1β were not different from baseline after challenge with either buffered saline or corn dust extract (data not shown).

Inhalation of corn dust extract resulted in a profound inflammatory response in the lower respiratory tract characterized by a neutrophilic alveolitis with enhanced cytokine production and release. Significantly higher cell concentrations in the BAL fluid were observed after inhalation of corn dust extract than after buffered saline (Figure 2). This increase in cellularity was comprised primarily of neutrophils. Importantly, all cytokines measured (IL-1β, IL-1RA, IL-6, IL-8, and TNF-α) in the BAL fluid were significantly higher after inhalation of corn dust extract than after buffered saline (Figure 3). However, the concentrations of 15-HETE, PGE_2, LTB_4, and histamine in BAL fluid were not different following challenge with corn dust extract versus buffered saline (data not shown). Moreover, the concentration of mRNA for IL1-β, IL-1RA, IL-6, IL-8, and TNF-α was significantly greater in BAL cells obtained following inhalation of corn dust extract than in those harvested after inhalation of buffered saline (Figure 4).

The inflammatory response appeared to be related to the physiologic response. When data from both challenges (buffered saline and corn dust extract) were analyzed together, the maximal percentage decrease in FEV_1 correlated significantly with the concentration of neutrophils in the BAL fluid ($r = 0.68$, $p = 0.001$, Figure 5), as well as with the concentration of TNF-α ($r = 0.39$, $p = 0.03$), IL-1β ($r = 0.05$, $p = 0.005$), IL-1RA ($r = 0.65$, $p = 0.001$), IL-6 ($r = 0.70$, $p = 0.001$), and IL-8 ($r = 0.62$, $p = 0.001$) in the BAL fluid. When these analyses were restricted to the data obtained from challenges with only corn dust extract, however, none of these relationships remained significant. These findings suggest that although a relationship exists between the presence of inflammation in the lower respiratory tract and the development of airflow obstruction, within an established inflammatory response, the degree of inflammation in the lower respiratory tract is not clearly associated with the degree of airflow obstruction.

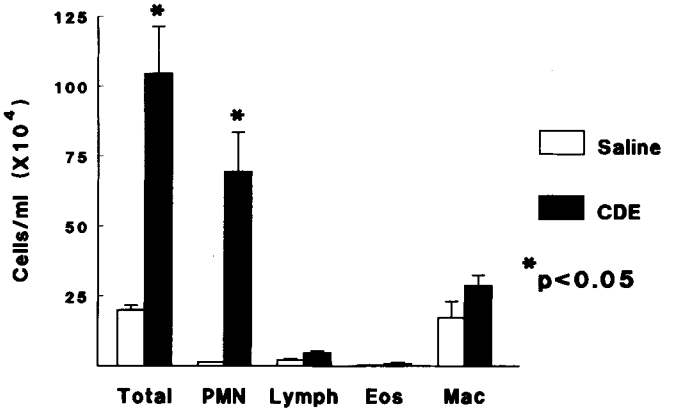

Figure 2. Bronchoalveolar lavage cell concentration (± SEM) after inhalation challenge. CDE = corn dust extract; saline = buffered saline.

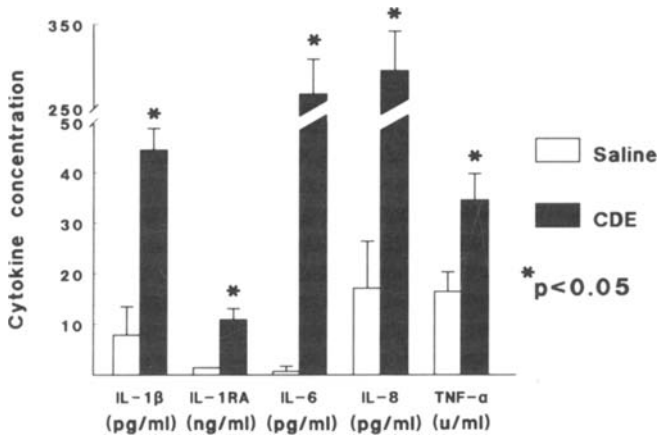

Figure 3. Cytokine concentration (± SEM) in BAL fluid after inhalation challenge. CDE = corn dust extract; saline = buffered saline.

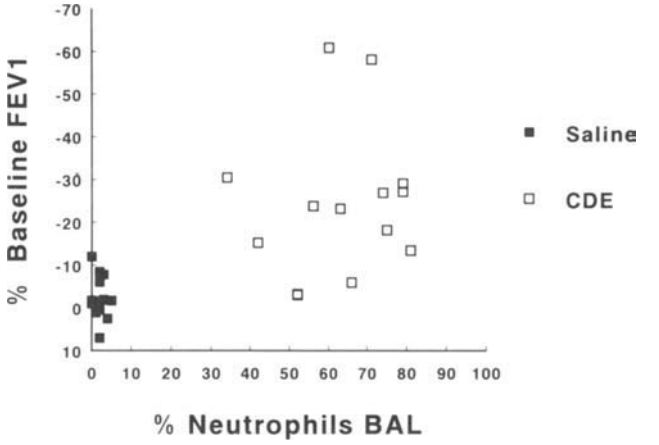

Figure 5. Scatter plot of maximal percentage decrease in FEV_1 after inhalation challenge with saline and corn dust extract (CDE) versus percentage of neutrophils in the bronchoalveolar lavage (BAL) fluid ($r = 0.68$, $p = 0.001$).

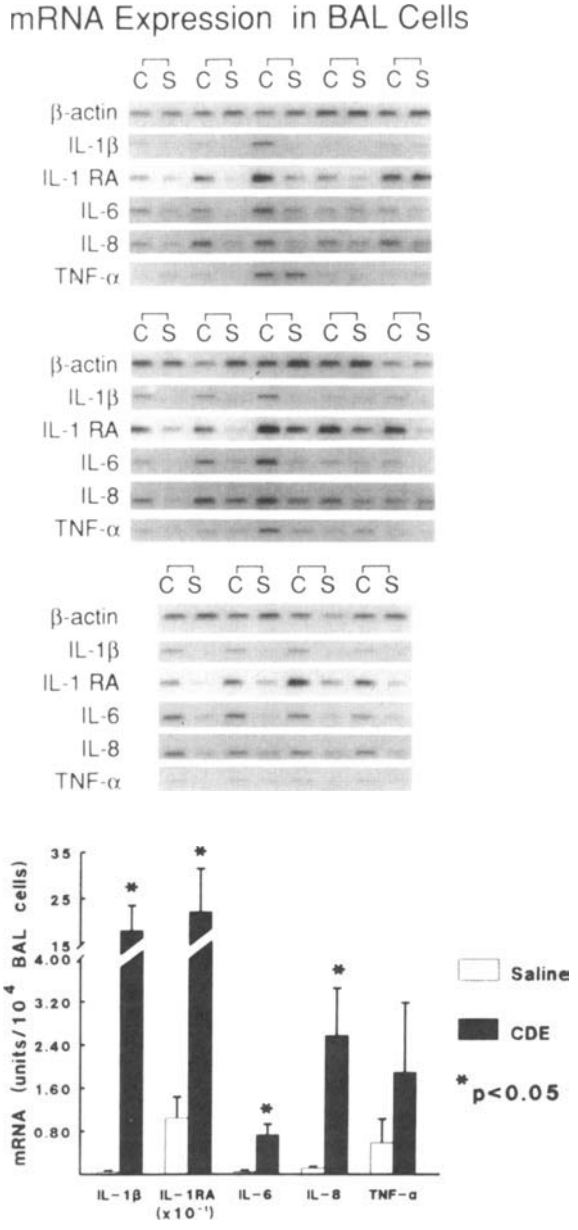

Figure 4. Gel and densitometry of bronchoalveolar lavage cell mRNA (± SEM) for TNF-α, IL-1β, IL-1RA, IL-6, and IL-8 after inhalation challenge. C and CDE = corn dust extract; S and saline = buffered saline.

DISCUSSION

Our findings indicate that inhalation of grain dust extract results in acute airflow obstruction, fever, and peripheral leukocytosis. These physiologic events are accompanied by a profound inflammatory response in the lower respiratory tract. These physiologic and biologic responses to inhaled corn dust extract are not dependent on preexisting asthma or the presence of atopy. Our results suggest that classic immunologic mechanisms do not initiate this inflammatory response. Furthermore, the airway response to grain dust extract appears to represent an acute inflammatory response to an inhaled toxin, such as endotoxin. Importantly, the alveolar macrophage and its specific cytokines may play a significant role in neutrophil recruitment.

Immune mechanisms may be operative in some grain handlers who develop airflow obstruction (39, 40), but several lines of evidence indicate that the physiologic response to grain dust extract is primarily mediated by an acute nonimmunologic inflammatory response in the lower respiratory tract. First, although a large proportion of grain handlers develop airway disease (4), atopic subjects tend to leave the grain-handling industry because they have a higher propensity for adverse symptoms in association with grain dust exposure than nonatopic workers (1). Despite this observation, atopic status and the presence of specific antibodies have not been consistently associated with either acute (11) or chronic (14) airway responses to inhaled grain dust. Second, the magnitude of both the acute and chronic pulmonary function deterioration seen in grain handlers is clearly related to the dose of airborne dust inhaled (7, 10, 12) but not dependent on the atopic status. Third, human inhalation studies have demonstrated that grain dust can induce airflow obstruction in previously unexposed individuals (41); grain dust-induced airflow obstruction occurs within 30 min of exposure (41–43), and this airway response is dependent on the inhaled dose of grain dust (41–43). Finally, *in vitro* studies have demonstrated that grain dust can induce alveolar macrophages to release neutrophil chemotactic factors (22) and interleukin-1 (44), and animal studies have shown that inhaled grain dust causes a profound neutrophilic response in the lower respiratory tract (22, 45). Importantly, humans challenged with aerosols of grain dust extract develop airflow obstruction (46) and rapidly accumulate neutrophils in the upper and lower respiratory tract (47). Results from the current study support and extend

these observations. We found that all subjects challenged with corn dust extract developed airflow obstruction in association with a profound influx of neutrophils in the lower respiratory tract, with a marked increase in cytokine production and release. Importantly, histamine levels were unmeasurable, and the immunologic response observed in atopic asthmatic subjects was absent. In aggregate, these findings strongly suggest that a specific toxin, capable of inducing neutrophil chemotaxis in previously exposed and also unexposed individuals, is responsible for the development of airway inflammation and airflow obstruction in grain handlers.

Although several components of grain dust may be responsible for initiating the physiologic and biologic response (22, 48, 49), it is very likely that endotoxin is the principal component of grain dust responsible for the development of airway inflammation and airflow obstruction (22, 50). Endotoxin has been implicated as an important inflammatory component of other organic dusts, including cotton dust, in which the airborne endotoxin level has been shown to correlate more closely with the severity of airflow obstruction than the airborne dust level (51). Furthermore, inhalation challenge with endotoxin results in airflow obstruction, fever, myalgias, peripheral leukocytosis (46, 52), and neutrophilic alveolitis (50, 53). These responses were seen in our study subjects following inhalation of corn dust extract. Endotoxin has also been shown to induce the release of TNF-α (54, 55), IL-1β (56, 57), IL-1RA (58, 59), IL-6 (60, 61), and IL-8 (62) from macrophages. Each of these cytokines was found in higher concentrations in the BAL fluid of our subjects after inhalation of corn dust extract. Leakage of these cytokines into the alveolar space was unlikely because TNF-α and IL-1β were not elevated in the serum of our subjects following inhalation of corn dust extract. Moreover, compared with the response to buffered saline, mRNA for all these cytokines was found to be increased in the cells obtained by BAL after inhalation of corn dust extract. As with the cytokines, these specific messages have been shown to be increased in macrophages in responses to endotoxin exposure (63, 64). Thus, although corn dust extract is clearly proinflammatory, this investigation was not designed to determine the independent effects of endotoxin in the development of grain dust-induced airway injury. The hypothesis that endotoxin in corn dust may explain much of its biologic activity warrants further investigation.

Our results suggest that inhalation of grain dust stimulates alveolar macrophages to upregulate mRNA for specific inflammatory cytokines and to produce and release those chemotactic cytokines. The presence of neutrophils in these areas of cytokine production and release suggests that alveolar macrophages may play a pivotal role in initiating and amplifying the inflammatory response. Admittedly, our methods preclude the identification of the macrophage as the primary cell responsible for the amplification of mRNA specific for proinflammatory cytokines. Interference with any of these processes could potentially reduce the severity of the inflammation. Inhibition of TNF-α (54, 65), IL-6 (66), or IL-8 (64) release by macrophages and interference with the proinflammatory effects of these cytokines are potential points in the cytokine network at which interventions may be possible. Therefore, such agents as pentoxifylline (67), inhaled glucocorticoids (54), pentamidine (68), and IL-1RA (69) may prove to be effective modulators of the physiologic and inflammatory response to inhaled grain dust.

This model does not simulate the exposure of grain workers to grain dust precisely, but it has allowed us to characterize the physiologic and biologic response to inhalation challenge with corn dust extract in 15 nonatopic nonsmoking grain handlers. We have shown that inhalation challenge with a large dose of corn dust extract alters pulmonary physiology in association with a local and systemic biologic response. Cellular, biochemical, and molecular markers of this response have been elucidated, providing new insight into the mechanism of the resultant inflammatory response and allowing speculation regarding possible intervention. Importantly, this model and these biomarkers provide a useful basis for further investigation of the effects of individual components of grain dust (such as endotoxin, mycotoxins, and individual proteins), the effects of different doses, and the efficacy of specific interventions. This approach will allow us to investigate the early inflammatory processes in the lower respiratory tract that are responsible for the development of environmentally induced asthma and bronchitis.

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