# The Kinetics of Grain Dust–induced Inflammation of the Lower Respiratory Tract

DANIEL C. DEETZ, PAUL J. JAGIELO, TIMOTHY J. QUINN, PETER S. THORNE, SHARI A. BLEUER, and DAVID A. SCHWARTZ

Departments of Internal Medicine and Preventive Medicine and Environmental Health, The University of Iowa College of Medicine, Iowa City, Iowa

**To characterize the kinetics of grain dust–induced airflow obstruction and inflammation in the lower respiratory tract, we performed serial spirometry and bronchoalveolar lavage (BAL) in human subjects and whole-lung lavage in mice following a single inhalation exposure to comparable dosages of corn dust extract (CDE). Following inhalation of CDE, our human study subjects developed significant airflow obstruction 10 min postexposure which persisted for 48 h. Human subjects and mice had similar acute and persistent changes in lavage cellularity after exposure to CDE. A profound increase in the concentration of lavage neutrophils was present in the initial postexposure lavage in both human subjects and mice. This increase persisted for 96 h in human subjects and 48 h in mice. Small but significant increases in lavage macrophage concentration were present 48 h postexposure in human subjects and at 96 h postexposure in mice. Inhalation of CDE resulted in a significant increase in the concentration of proinflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) in the lavage fluid of both human subjects and mice. Similarly, significant increases in lavage concentrations of IL-8 in humans and macrophage inflammatory protein-2 (MIP-2) in mice occurred after inhalation of CDE. The lavage concentration of all measured proinflammatory cytokines returned to baseline levels by 168 h in humans and 96 h in mice. These findings suggest that a single inhalation challenge of CDE results in airflow obstruction and lower respiratory tract inflammation that may last for several days. These physiologic and inflammatory responses appear to be self-limited with no evidence of persistent injury 1 wk after the inhalation challenge. Moreover, the inflammatory response in the lower respiratory tract after inhalation of grain dust is similar in human subjects and mice, suggesting that the mouse may be an appropriate model for further investigation of grain dust–induced inflammation. Deetz DC, Jagielo PJ, Quinn TJ, Thorne PS, Bleuer SA, Schwartz DA. The kinetics of grain dust–induced inflammation of the lower respiratory tract. AM J RESPIR CRIT CARE MED 1997;155:254–259.**

Occupational or environmental exposure to grain dust has been shown to result in an increased prevalence of both acute and chronic respiratory diseases, represented primarily by airflow obstruction and airway inflammation. Workshift decrements in $FEV_1$ ($\leq$ 10%) have been shown to occur in 4 to 11% of grain elevator workers (1–3) and the prevalence of chronic bronchitis among nonsmoking grain workers has been noted to range from 15 to 37% (4). Furthermore, longitudinal studies of lung function among grain workers have shown an accelerated decline in airflow (5–7). Interestingly, the development of chronic airflow obstruction in grain workers appears to be associated with acute grain dust–induced changes in airflow (5–7), suggesting that investigation of the acute inflammatory response may provide insight into the mechanisms of chronic airflow obstruction.

The pathophysiology of acute grain dust–induced lung disease does not appear to be mediated through classic immunologic mechanisms. Acute airflow obstruction is not associated with either prior exposure (8), atopic status (2, 8), or presence of antibodies (2). Grain dust has been shown to activate alveolar macrophages and cause a neutrophilic infiltration of the lower respiratory tract (9, 10). This inflammatory response is thought to be mediated by the acute production and release of proinflammatory cytokines (11). There is growing evidence suggesting that endotoxin is the primary constituent in grain dust responsible for inflammation in the lower respiratory tract. Respiratory symptoms and airflow obstruction among grain handlers are strongly related to the concentration of endotoxin in the work place bioaerosol (12). Endotoxin responsiveness appears to be required for the development of the inflammatory process following inhalation of grain dust. Mice that are hyporesponsive to endotoxin through genetic or acquired means have a diminished inflammatory response to grain dust (13). Additionally, reduction in the endotoxin concentration in grain dust results in a substantial decline in grain dust–induced inflammation (14).

Although the initial events of the acute inflammatory response

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Correspondence and requests for reprints should be addressed to David A. Schwartz, M.D., M.P.H., Pulmonary Diseases, Critical Care, Occupational Medicine Division, Department of Internal Medicine, The University of Iowa College of Medicine, Iowa City, IA 52242–1081.

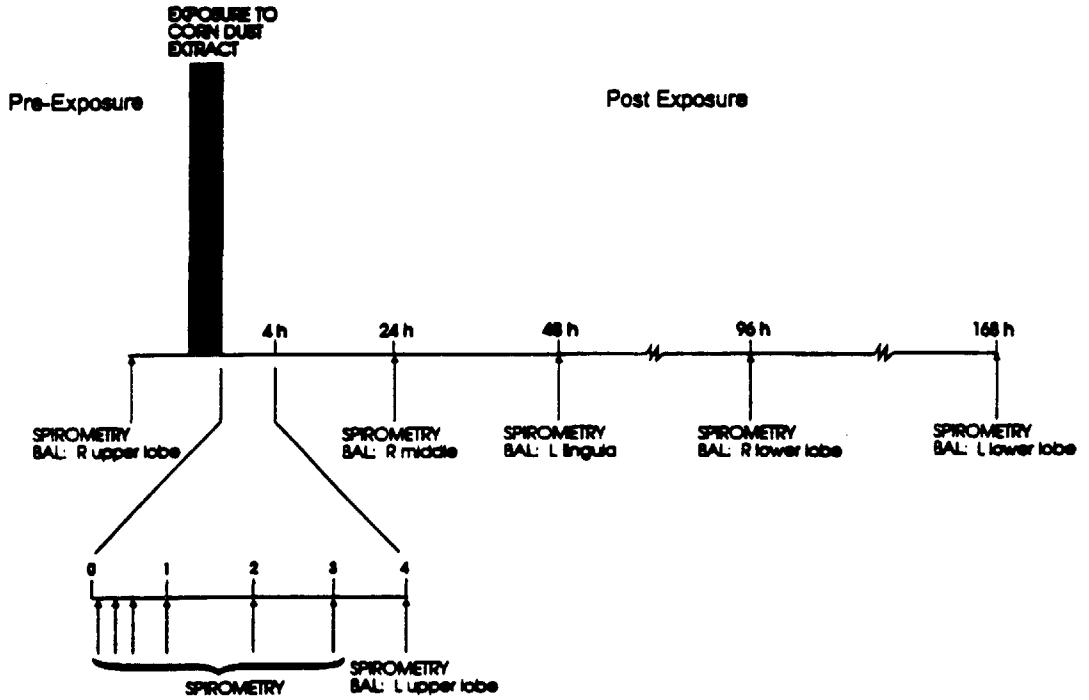


*Figure 1.* Protocol for serial pulmonary function testing and bronchoscopy in relation to inhalation exposure of CDE for human subjects.

have been investigated, little is known about the time course of the physiologic or inflammatory effects in the lower respiratory tract after inhalation of grain dust. In this study, we examined the kinetics of grain dust–induced inflammation in both human subjects and in a murine model. Physiologic correlates of the inflammatory response were obtained in human subjects. Our hypothesis was that the acute inflammatory response to inhaled grain dust is a self-limited event and that the kinetic profiles of the inflammatory response would be similar in both humans and mice. Our results indicate that a single inhalation challenge of corn dust extract produces rapid changes in airflow and inflammation of the lower respiratory tract that may persist for several days but are not detectable 1 wk after exposure. Importantly, the kinetics and constituents of the inflammatory response induced by the inhalation of grain dust extract were similar in humans and mice. The physiologic and inflammatory changes following a single inhalation of CDE appear to be self-limited, without evidence of persistent injury.

## METHODS

### General Experimental Protocol

Human subjects were admitted to the University of Iowa General Clinical Research Center (GCRC) and a standard protocol was followed for all cases (Figure 1). To determine the time course of physiologic and inflammatory effects of inhaled corn dust extract (CDE), human subjects underwent preexposure spirometry and bronchoscopy. Aerosolized CDE was then delivered by nebulizer until a target dose was achieved. Delivery of CDE occurred over 55 to 90 min (mean 74 min) in our human subjects. Postexposure spirometry and bronchoscopy were then performed in serial fashion. Mice were exposed for 4 h to aerosolized CDE in a 75-L glass exposure chamber. After exposure, mice were euthanized at selected time points and underwent whole-lung lavage (Figure 2). Comparisons were performed for measures of airflow obstruction in human subjects and inflammation in both mice and human subjects at different time points after exposure. The conclusion of the inhalation exposure was arbitrarily designated as time zero for both human subjects and mice. All time points for this investigation reflect elapsed time from the end of exposure.

### Human Subjects

Subjects were recruited by newspaper advertisement. Subjects were required to be healthy never-smokers, taking no medications, with no history of agricultural work. Moreover, they were required to not have asthma by history, have a negative skin test response to a standard panel of aeroallergens, have normal baseline measures of lung function and chest radiograph, and have a negative response to an abbreviated histamine challenge. Five subjects were recruited for this investigation. The mean age of our subjects was 39 yr (range, 24 to 49) and all were male. All subjects gave informed written consent for their participation in this investigation. This study was approved by the University of Iowa Institutional Review Board for studies involving human subjects and the Institutional Animal Care and Use Committee.

### Animals

Endotoxin-sensitive (C3H/HeBFEJ) male mice (Jackson Laboratories, Bar Harbor, ME) were obtained at 6 wk of age and used within 2 wk. A total of 108 mice were used for this investigation. Six mice were lavaged at each of the six time points. Mean weight at the time of exposure was 24.6 ± 1.3 g. Mice were housed within our institution's rodent vivarium. All animal care and housing requirements set forth by the National Institutes of Health Committee on Care and use of Laboratory Animal Resources were followed, and animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee. Mice were maintained in wood chip bedding (Northeastern Product, Warrensburg, NY) with food (Formulab Chow 5008; PMI, Richmond, IN) and water supplied *ad libitum*.

### Human Exposure Apparatus

The solutions were administered via a DeVilbiss 646 nebulizer and DeVilbiss dosimeter (DeVilbiss Health Care Inc., Somerset, PA). A standardized protocol was followed which has been previously described (11). Subjects inhaled enough solution of corn dust extract to achieve a dose of

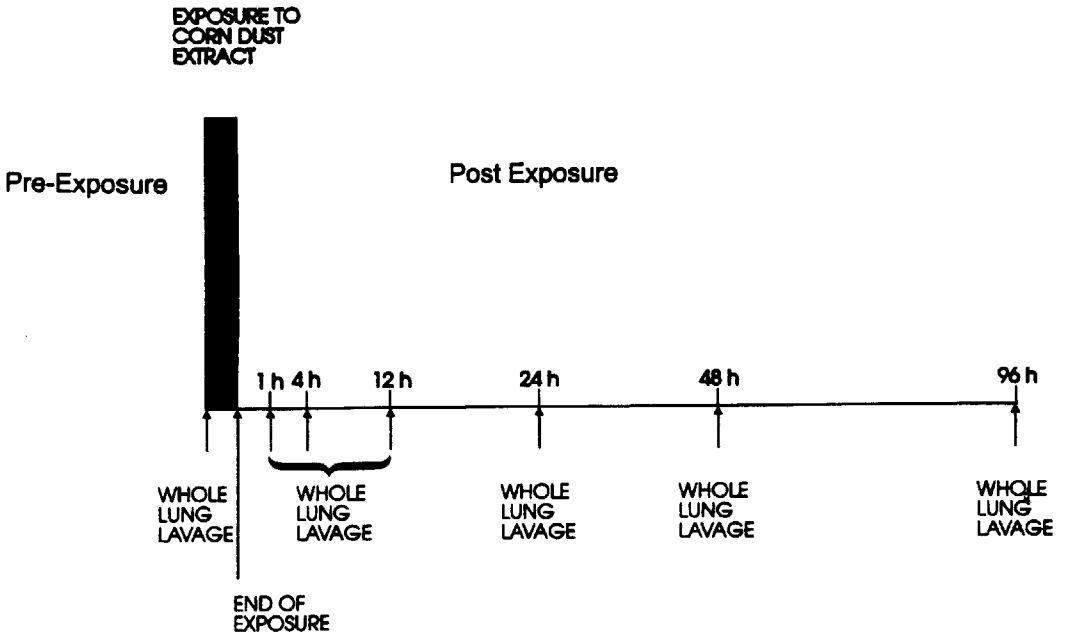


*Figure 2.* Protocol for serial lung lavage in relation to inhalation exposure of CDE for mice.

0.4 μg endotoxin/kg body mass. This does of CDE was estimated to expose a study subject to the amount of endotoxin that a typical agricultural worker would inhale during an 8-h workshift.

### Murine Exposure Apparatus

Corn dust extract and endotoxin aerosols were generated into a glass 75-L exposure chamber using a PITT Number 1 nebulizer. High-efficiency particulate absolute (HEPA)-filtered air was supplied to the nebulizer at flow rates of 14 L/min. Mixing within the chamber was aided by a magnetically coupled rotor. The chamber atmosphere was ventilated at 11 changes/hour. Endotoxin concentrations were determined by sampling the total chamber outflow. Particle size distributions were determined with an Aerodynamic Particle Sizer (TSI, Inc., St. Paul, MN) and gravimetrically with a Marple personal cascade impactor and Mylar media by sampling within the exposure chamber. Airborne endotoxin concentration in the murine exposure chamber was 8.3 μg/m³. Mass median aerodynamic diameter for CDE was 1.2 μm. The murine exposure resulted in an estimated dose of 0.5 μg endotoxin/kg body mass.

### Corn Dust Extract Preparation

The corn dust for these studies was obtained from the air filtration system at an eastern Iowa grain handling facility. The collected dust was stored at 4° C. Extracts were prepared by methods described previously (13). Sterility of the extract was confirmed by culture on trypticase soy agar at 35° C and 52° C, MacConkey's agar at 35° C, and malt extract agar at 25° C.

### Chemicals

Sterile pyrogen-free saline (pfs) was obtained from Baxter Medical Laboratories (Deerfield, IL). Sterile pyrogen-free water (pfw) was purchased from the University of Iowa Pharmacy Service.

### Endotoxin Assay for Animal Inhalation Studies

The endotoxin concentrations of grain dust extracts and aerosols were assayed using the chromogenic *Limulus* amebocyte assay (QCL-1000; Whittaker Bioproducts, Walkersville, MD) with sterile, pyrogen-free labware and a temperature-controlled microplate block and microplate reader (405 nm).

### Pulmonary Function Testing

The pulmonary function tests consisted of serial spirometry using a spirometer (Spirotech S-600; Graseby Anderson, Atlanta, GA). These maneuvers were performed using standard protocols and the American Thoracic Society guidelines (15). Spirometry was performed before exposure to establish baseline pulmonary functions and was performed at 11 predetermined time points after inhalation of CDE in our human subjects (Figure 1).

### Human Bronchoalveolar Lavage

Bronchoscopy was performed six times (1 h before exposure and at 4, 24, 48, 96, and 168 h after the exposure) for each human subject using a standard protocol (Figure 1). Subjects were premedicated with atropine, morphine, and midazolam for comfort and safety. An Olympus P-10 (1.5 mm channel; Olympus, Lombard, IL) bronchoscope was introduced through the oral cavity. The vocal cords, carina, and mainstem bronchi were anesthetized with 3 ml of 2% lidocaine. The bronchoscope was gently passed through the trachea, and wedged into a subsegmental bronchus where 20 ml of 37° C pfs was introduced. 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. Analysis of inflammatory changes in the airway fraction was not performed. A different segmental bronchus was used for each of the six BALs that were performed over a 1-wk period. A standardized protocol was followed to select the segmental bronchus for each of the six BAL procedures.

### Animal Lung Lavage

After the inhalation exposure, mice were killed by cervical dislocation at 1, 4, 12, 24, 48, or 96 h. Control mice exposed to ambient air only were lavaged to establish a baseline or preexposure measure of inflammation. The chest was opened and lungs were lavaged *in situ* via PE-90 tubing cannulated into the exposed trachea. A pressure of 25 cm $H_2O$ was used to lavage the lungs with 6.0 ml sterile pfs.

### Treatment of Bronchoalveolar and Whole-lung Lavage Fluid

Our previously established method of processing the sample was used

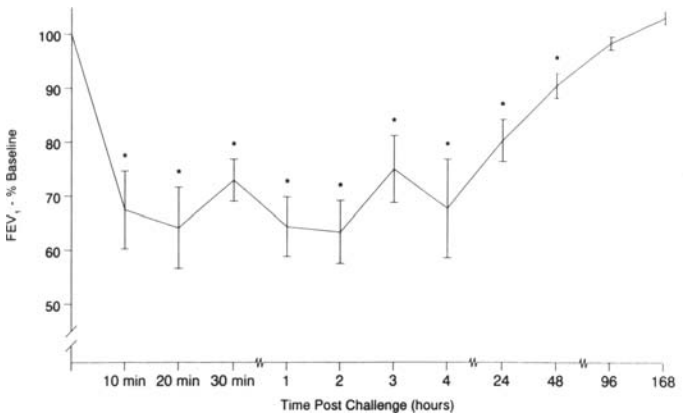


*Figure 3.* Change in $FEV_1$ in human subjects as a percentage of preinhalation values after a single inhalation challenge of CDE. *p < 0.05 compared with pre-exposure values.

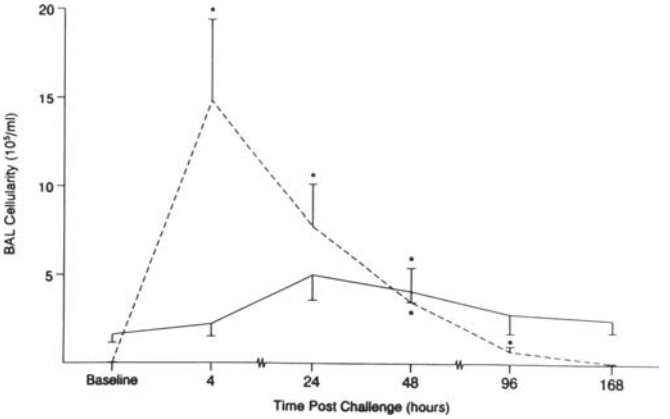


*Figure 4.* The concentration of BAL neutrophils (*dashed lines*) and macrophages (*solid lines*) after a single inhalation challenge of CDE in human subjects. *p < 0.05 compared with pre-exposure values.

(16). Cell Concentrations were performed using a hemocytometer and cytospin preparations.

### Measurement of Cytokines in Lavage Fluid

Tumor necrosis factor-alpha (TNF-α) bioactivity was measured in both human and mouse lavage specimens with a cytotoxicity assay using the TNF-α-sensitive L929 mouse fibroblast cell assay. Standard, commercially available ELISA systems were used for measurements of human interleukin-6 (IL-6), interleukin-8 (IL-8) (R&D Systems, Minneapolis, MN), and murine IL-6 (Endogen Inc., Boston, MA). Macrophage inflammatory protein-2 (MIP-2) was measured using a rat MIP-2 ELISA (Biosource International, Camarillo, CA) which contains antibodies cross-reactive to murine MIP-2. A standard curve was generated using recombinant murine MIP-2 from Chiron Corporatio (Emery, CA).

### Statistical Analysis

Comparisons were performed to investigate the kinetics of the physiologic and inflammatory response to inhaled grain dust in humans and mice. Paired comparisons for measures of inflammation were performed using preexposure data in human subjects. For mice, unpaired comparisons were performed using data from unexposed mice. Nonparametric statistics including the Mann-Whitney U test and Wilcoxon matched-pairs signed rank test (17) were used to assess the statistical significance of these comparisons.

## RESULTS

Inhalation exposure to CDE rapidly resulted in significant airflow obstruction (Figure 3). Ten minutes after exposure, $FEV_1$ and FVC were reduced from preinhalation values by 31% and 26%, respectively. Maximal reductions in airflow occurred 2 h postexposure with $FEV_1$ and FVC reductions of 36% and 31% from their baseline values. Surprisingly, significant decrements in airflow persisted for 48 h.

Similar inflammatory changes in the lower respiratory tract were observed in human subjects and mice following exposure to CDE (Figures 4 and 5). Moreover, the kinetic profiles of cellular changes in the lavage fluid were remarkably similar between human subjects and mice. A profound increase in the concentration of polymorphonuclear neutrophils (PMNs) was noted in the BAL fluid of our human subjects and whole-lung lavage specimens in mice. The concentration of PMNs peaked at 4 h postexposure in our human subjects and peaked at 1 h postexposure in the mice, representing the first postexposure lavage collections in each group. Elevations in lavage neutrophil concentration persisted for 96 h in humans and 48 h in mice. A small but statistically significant elevation in lavage macrophage concentration was present 48 h postexposure in our human subjects and at 96 h postexposure in mice. There were no significant changes in the lavage concentrations of eosinophils or lymphocytes in our human subjects or mice at any time point (data not shown).

Inhalation of CDE resulted in significant increases in proinflammatory cytokine concentration in the lavage fluid of human subjects and mice (Figures 6 and 7). Concentrations of inflammatory mediators TNF-α and IL-6 were at maximal levels in the initial postexposure lavage in both humans and mice. Lavage TNF-α concentrations remained significantly elevated for 12 h in humans and for 24 h in mice. Lavage IL-6 concentrations remained significantly elevated for 96 h in human subjects and for 24 h in mice. In humans, BAL concentrations of IL-8 were highest in the initial postexposure lavage, similar to TNF-α and IL-6. In mice, however, lavage MIP-2 concentrations peaked at 24 h. Significant elevations in IL-8 and MIP-2 concentrations persisted for 48 h in humans and mice, respectively.

## DISCUSSION

Our results demonstrate that a single inhalation exposure to CDE

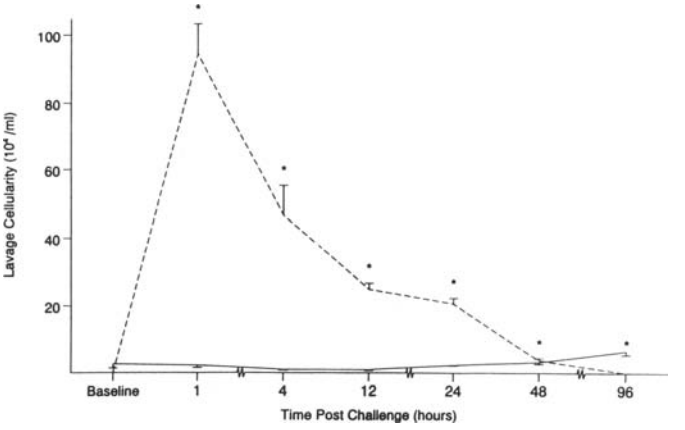


*Figure 5.* The concentration of lavage neutrophils (*dashed lines*) and macrophages (*solid lines*) after a single inhalation challenge of CDE in mice. Baseline values represent lavage cellularity in unexposed mice. *p < 0.05 compared with unexposed mice.

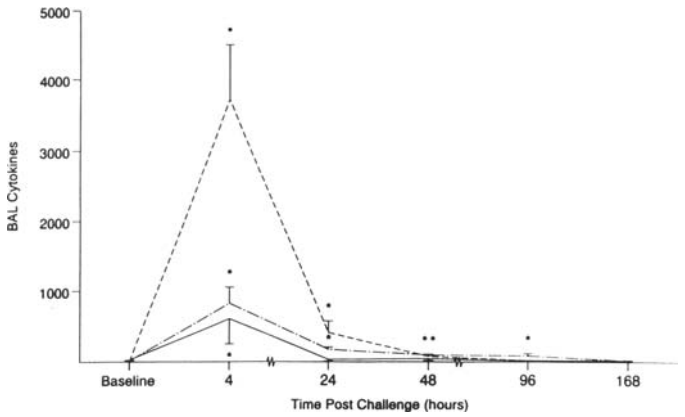


*Figure 6.* Concentration of BAL cytokines TNF-α (*solid lines*), IL-6 (*dashed lines*), and IL-8 (*hatched lines*) in human subjects after a single inhalation challenge of CDE. *$p < 0.05$ compared with pre-exposure values.

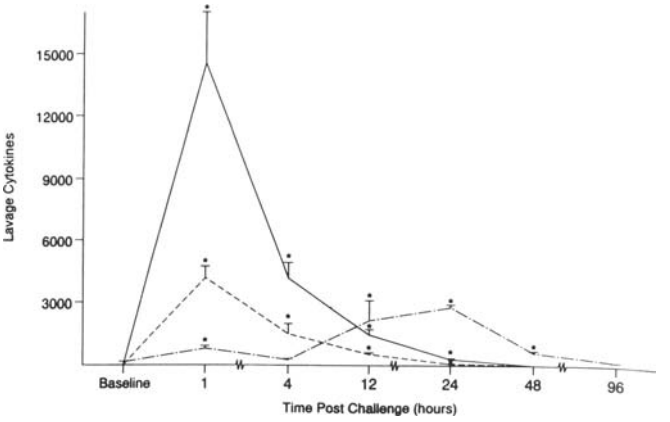


*Figure 7.* Concentration of lavage cytokines TNF-α (*solid lines*), IL-6 (*dashed lines*), and MIP-2 (*hatched lines*) in mice after a single inhalation challenge of CDE. Baseline values represent lavage cellularity in unexposed mice. *$p < 0.05$ compared with unexposed mice.

results in a rapid physiologic and inflammatory response that may persist for several days but is ultimately self-limited. Significant decrements in airflow were present in our human subjects immediately after inhalation challenge and persisted for 48 h. Similarly, peak measures of inflammation in our human subjects and mice were present immediately after the exposure. This was observed for both cellular markers of inflammation and proinflammatory cytokines. Importantly, there was no evidence of physiologic or inflammatory changes present after 96 h, suggesting that resolution of the acute inflammatory response was complete without evidence of persistent injury. Moreover, our results have demonstrated similar inflammatory responses in mice and humans following inhalation of grain dust, suggesting that the mouse model provides an opportunity to study the pathogenesis of grain dust–induced lung disease in humans.

Our results support and extend the findings of other investigators who have examined the time course of inflammation following inhalation of either grain dust or lipopolysaccharide (LPS). For instance, hamsters developed a marked increase in the concentration of lavage PMNs that lasted for 24 h after a single inhalation challenge of LPS (18), and humans were found to have a peripheral neutrophilia that persisted for 9 h and an increase in the concentration of BAL PMNs that was evident at 24 h after inhalation of grain sorghum dust extract (19). Interestingly, human volunteers exposed to swine dust were found to have increases in concentration of TNF-α and IL-6 in the peripheral blood (20). The increase in TNF-α was observed between 3 and 5 h after exposure and the increase in IL-6 was observed between 4 and 11 h after exposure. These results, in conjunction with our findings, indicate the persistence of an inflammatory response that is initiated by grain dust or LPS and primarily involves neutrophils and proinflammatory cytokines.

Although our results suggest that a single exposure to grain dust results in self-limited airway injury, one could hypothesize that recurrent episodes of airway injury may result in persistent airway disease. Epidemiologic studies have demonstrated an association between the acute physiologic response to grain dust and the development of chronic airflow obstruction (5–7). Moreover, among cotton workers (21) and agricultural workers (22), progressive airflow obstruction was found to be associated with across-shift changes in $FEV_1$. Although our results suggest that resolution of acute airway injury is complete, repeated exposures to grain dust may result in chronic airway inflammation and remodeling. The persistent inflammatory and anatomic changes may place individuals at a higher risk of developing chronic airway disease and accelerate the decline in lung function.

Our study suggests that the murine animal model of grain dust–induced lung disease may be appropriate for investigation of the acute inflammatory response to inhaled grain dust. The inflammatory response to inhaled grain dust was similar in both our human and animal model. Although physiologic correlates of the inflammatory response were not measured in mice, measurements of lavage cellularity and proinflammatory cytokines were similar to those observed in humans. Moreover, the kinetics of the inflammatory response was very similar for most of the inflammatory mediators measured in the lavage fluid. This animal model of grain dust–induced lung disease may be useful for further identifying pathogenic mechanisms of grain dust–induced lung disease as well as investigating experimental agents to prevent or treat grain dust–induced lung disease.

Small differences in measures of the acute inflammatory response to inhaled CDE were observed between human subjects and mice. Maximal values of BAL IL-8 occurred 4 h after exposure in humans while maximal values of lavage MIP-2 occurred at 24 h in mice. Despite this difference, significant elevations of MIP-2 and IL-8 lavage concentrations persisted postexposure for equivalent periods of time. Additionally, the concentration and time course of lavage neutrophils was very similar for both humans and mice. IL-8 and MIP-2 are members of the same supergene family, share significant sequence homology, and are potent chemoattractants for neutrophils (23). Although IL-8 and MIP-2 are structurally and functionally similar, some differences may exist that result in the slightly discordant time courses. Synthesis of IL-8 has been shown from alveolar macrophages, epithelial cells, fibroblasts, and monocytes (24, 25). Similarly, alveolar macrophages, fibroblasts, and epithelium have been shown to produce MIP-2 (26). More recently however, MIP-2 messenger RNA (mRNA) has also been shown to localize to neutrophils by *in situ* hybridization (27). As MIP-2 has been isolated only recently, the functional characteristics of MIP-2 are not yet elucidated to allow complete comparison with human IL-8.

A potential limitation of this investigation is the possible effect that prior BAL may have on the inflammation assessed by subsequent BAL. While our animal studies were performed using one lavage for each animal, our human studies required six separate bronchoscopies for each subject. If prior BAL affects the inflammatory response in subsequent BAL, our data would be confounded by the study protocol. However, at least three lines

of reasoning indicate that our data are valid. First, previous studies (28, 29) indicate that prior bronchoscopy does not significantly alter subsequent BAL cellularity in lobes not previously lavaged. However, this is somewhat controversial since it has also been shown that BAL in one segment of the lung was associated with neutrophil recruitment to nonlavaged lung segments (30). Second, our data in humans indicate that the BAL results from one subsegmental bronchus prior to CDE inhalation are virtually identical to the BAL cells and cytokines from a different subsegmental bronchus after five prior lavages. Third, the inflammatory response we observed in humans parallels that which we observed in mice subjected to only one lavage. In aggregate, previous studies (28, 29) and our results strongly suggest that the kinetics of the inflammatory response is not altered by repeated bronchoscopic procedures, but instead is driven by the inhalation of grain dust.

In summary, our findings suggest that a single inhalation challenge of CDE results in airflow obstruction and lower respiratory tract inflammation that may last for several days. These physiologic and inflammatory responses appear to be self-limited with no evidence of persistent injury 1 wk after the inhalation challenge. Moreover, the inflammatory response in the lower respiratory tract after inhalation of grain dust is similar in human subjects and mice, suggesting that the mouse may be an appropriate model for further investigation of grain dust–induced inflammation.

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