GRAIN DUST, ENDOTOXIN, AND AIRFLOW OBSTRUCTION

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Abstract

Occupational and environmental exposure to grain dust can cause a spectrum of clinical syndromes, including asthma, acute and chronic changes in airway reactivity, grain fever, bronchitis, and progressive irreversible airflow obstruction. Exposure-response studies have shown that declines in lung function across the workshift are directly related to higher ambient concentrations of grain dust. Although the pathogenesis of grain dust-induced airway disease is not known, several lines of evidence indicate that the physiologic response to grain dust is primarily mediated by an acute non-allergic inflammatory response in the lower respiratory tract. Our results indicate that grain dust-induced lung disease is primarily caused by endotoxin and that the alveolar macrophage, airway epithelia, and specific proinflammatory cytokines appear to be particularly important in the initial inflammatory response. Several lines of evidence will be presented supporting these findings. First, we will examine the physiologic and biologic response to inhaled grain dust and directly compare this response to the physiologic and biologic response to inhaled endotoxin. Second, we will present results from studies in which we have modified the concentration of endotoxin in the bioaerosol to determine the effect that this has on the inflammatory response to grain dust. Third, we will present data from studies in which we modified the ability of the host to respond to endotoxin and determined whether this affects the inflammatory response to grain dust. Finally, we will present findings from our genetic studies investigating the genetic basis for endotoxin hyporesponsiveness.

Introduction

Occupational and environmental exposure to grain dust can cause a spectrum of clinical syndromes, including asthma, acute and chronic changes in airway reactivity, grain fever, bronchitis, and progressive irreversible airflow obstruction (1). In North America alone, over 5 million agricultural workers are exposed to grain dust each year (1-4). Among grain handlers, the prevalence of work-shift changes in FEV_1 ($\geq$ 10% decline) varies between 3.9 and 11% (2-4). Grain fever, an acute illness consisting of fevers, chills, headache, myalgias, chest tightness, dyspnea, and a transient decline in airflow, has been reported in up to 25% of grain workers (5-7). Grain dust exposure causes seasonal decrements in airflow (8-10) and an accelerated longitudinal decline in FEV_1 (11-14). Chronic bronchitis occurs in a high prevalence (23 to 37%) of grain handlers (6, 11, 14, 15). The effect of cigarette smoking appears to be additive (12, 16), however, nonsmokers also develop grain dust-induced airway disease (15, 17, 18). Moreover, acute changes in airflow across either the work shift or work week are consistently associated with accelerated declines in lung function (9, 12, 13), and chronic occupational exposure to grain dust is associated with impaired lung function even among retired grain workers (19).

Exposure-response studies have shown that declines in lung function across the work shift are directly related to higher ambient concentrations of grain dust (4, 20). Importantly, 15 yr cumulative average dust exposures have been found to be directly related to chronic phlegm production, breathlessness on exertion, and lower spirometric measures of lung function (14). However, the concentration of inhaled endotoxin is strongly associated with the development of airflow obstruction among agricultural workers, including grain workers (21), swine confinement workers (22), poultry workers (23), and those exposed to cotton dust (24-26). Higher concentrations of endotoxin in grain facilities have been clearly associated with work-related symptoms of cough, chest tightness, and shortness of breath (21). Furthermore, we have recently shown that, among swine confinement operators and nonconfinement farmers, higher ambient concentrations of endotoxin are associated with longitudinal declines in measures of airflow (27). These studies suggest that the ambient concentration of endotoxin may be particularly important in the development of impaired lung function in grain workers as well as other workers chronically exposed to organic dusts.

Although the pathogenesis of grain dust-induced airway disease is not known, several lines of evidence indicate that the physiological response to grain dust is primarily mediated by an acute nonimmunologic inflammatory response in the lower respiratory tract. First, although atopy may play a role in selected cases of grain dust-induced airway disease, the atopic status and the presence of specific antibodies have not been consistently associated with acute (28-30) or chronic (12, 13, 16, 18, 31) airway responses to inhaled grain dust. Second, in vitro, grain dust can activate complement through the alternate and classical pathways (32-34); however, activation of complement does not appear to be important in the development of grain dust-induced airflow obstruction (29-30). Third, in vitro studies demonstrated that grain dust can induce alveolar macrophages to release neutrophil chemotactic factors (34) and interleukin (IL)-1 (35), and animal studies have shown that inhaled grain dust causes a profound neutrophilic response in the lower respiratory tract (34, 36). Finally, human inhalation studies have demonstrated that grain dust can induce airflow obstruction in previously unexposed individuals (30), grain dust-induced airflow obstruction occurs within 30 min of

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exposure (29, 30), and this airway response is dependent on the inhaled dose of grain dust (29, 30). Humans challenged with aerosols of grain dust extract will rapidly accumulate neutrophils in the upper and lower respiratory tract (37). In summary, these findings strongly suggest that a specific toxin or combination of agents capable of inducing neutrophil chemotaxis in previously exposed and unexposed individuals is responsible for the development of airway inflammation and airflow obstruction in grain handlers.

Endotoxin may be the principal component of grain dust responsible for the development of airway inflammation and airflow obstruction; however, little research has directly addressed this hypothesis. Endotoxin has been measured in similar concentrations from several of these vegetable dusts, including corn, wheat, oats, barley, and cotton (38). Moreover, recent findings indicate that, among those exposed to cotton dust, respirable levels of endotoxin are more predictive of respiratory impairment (symptoms and airflow obstruction) than are concentrations of airborne dust (25). Inhalational exposure of humans to endotoxin causes predictable decrements in airflow in previously unexposed subjects (39, 40). Inhalation of endotoxin can cause a profound inflammatory response in the lung that is best characterized by activation of alveolar macrophages and neutrophil recruitment to the lower respiratory tract. Animals challenged with sufficient doses of respirable endotoxin will acutely recruit neutrophils to the lung (interstitium, alveoli, and airway) (31, 41). Inhalation of endotoxin has also been shown to enhance the in vivo production of alveolar macrophage-derived platelet-activating factor (41) and results in a dose-dependent increase in the concentration of tumor necrosis factor (TNF)-α in bronchoalveolar lavage (BAL) fluid (42). The prominent role of the macrophage is further supported by in vitro studies that have shown that macrophages release neutrophil chemotactic factors (34), IL-1 (43), and TNF-α (44) when stimulated with lipopolysaccharide (LPS). Moreover, LPS-induced neutrophil chemotaxis appears to be dependent on the generation of TNF-α, IL-1β, and the IL-8 family of cytokines (42, 45, 46). In fact, animal studies have shown that inhalation of LPS results in a dose-dependent influx of neutrophils into the alveoli that can be partially inhibited by prior treatment with TNF-α-specific antibodies (42). These findings suggest that inhaled LPS initiates a complex interaction between alveolar macrophages and other inflammatory cells (primarily neutrophils), and this interaction appears to be mediated by specific cytokines. Importantly, the physiologic and biologic response to inhaled LPS appears to be similar to the response to inhaled grain dust.

In aggregate, these findings suggest that endotoxin is the principal component of grain dust responsible for the development of acute inflammation of the airway as well as the development of airflow obstruction. We have approached this overall hypothesis in three different ways. First, we have examined the physiologic and biologic response to inhalation of grain dust and directly compared that to the physiologic and biologic response to inhalation of endotoxin. Second, we altered the concentration of endotoxin in the bioaerosol to see whether we could alter the response to grain dust. Third, we altered the ability of the host to respond to endotoxin and determined whether the ability of the host to respond to endotoxin affected the inflammatory response to grain dust. In the following discussion, I will summarize the results from these exposure-response studies (47-49).

Physiologic and Biologic Response to Grain Dust in Comparison to Lipopolysaccharide

These exposure response studies in humans were performed on healthy volunteers. All of the individuals that were studied were pre-screened and had normal measures of lung function, no history of asthma or atopy, and were never cigarette smokers. These studies were performed as cross-over studies where each individual was exposed to two different agents; saline and to grain dust in the case of grain workers, and in the case of healthy volunteers, grain dust and similar concentrations of LPS. These exposures were separated by a three-week period of time and each person was compared to themselves. Water soluble extracts of corn dust were prepared using standard techniques (47). The concentration of endotoxin in the extract was measured and the exposure over a one-hour period of time was similar to the amount of endotoxin that a grain worker would be exposed to during an average work day in the grain industry.

Our results indicate that grain workers have a profound decrease in airflow when challenged with corn dust extract in comparison to the change in airflow when exposed to buffered saline (figure 1). This decline in airflow occurs within 30 minutes of exposure to corn dust extract. Importantly, the decline in airflow among grain workers was equivalent to what was observed in healthy volunteers following exposure to corn dust, suggesting that prior exposure to corn dust is not a prerequisite to develop airflow obstruction. Moreover, among healthy volunteers, the decline in airflow following exposure to LPS was equivalent to what was observed following exposure to corn dust (figure 1). Four hours after the inhalation challenge, fiberoptic bronchoscopy was performed on these study subjects. This procedure included bronchoalveolar lavage (BAL) of a subsegmented bronchus, an isolated airway lavage of the left main stem bronchus with a double balloon catheter, and an endobronchial brush biopsy of the distal trachea. This allows us to examine the inflammation in a bronchoalveolar unit, inflammation in a large airway, and the specific response of the airway epithelia. The BAL demonstrated a neutrophilic alveolitis within four hours of exposure to corn dust extract in grain workers and among healthy volunteers (figure 2). The neutrophilic alveolitis was also present following exposure to LPS. Marked increases in the concentration of proinflammatory (IL-1β,

IL-1RA, IL-6, IL-8, and TNF-α) cytokines were observed in the BAL fluid following inhalation of corn dust extract in both grain workers and healthy volunteers (figure 3). Similar increases in the concentration of these cytokines were seen in healthy volunteers following inhalation of either corn dust extract or LPS. Importantly, other mediators of inflammation, such as histamine, leukotriene B4, and prostaglandin E2 were not increased when study subjects were challenged with either corn dust extract or LPS. To assess whether inflammatory cells were actively responding to the inhalation challenge, we examined the concentration of mRNA in the BAL cells using RT-PCR (figure 4). Following exposure to corn dust extract an increased concentration of mRNA is clearly evident for IL-1β, IL-1RA, IL-6, IL-8. Although increases in the concentration of mRNA for TNF-α are present following inhalation of corn dust extract, these differences were not statistically significant and may reflect the four hour delay between exposure and BAL. The endobronchial lavage demonstrated that there is an active inflammatory response in the main conducting airways that consists primarily of an increase in the concentration of neutrophils and IL-8 with very little change in the concentration of lymphocytes, eosinophils, or epithelial cells (data not presented). Interestingly, examination of the airway epithelia that was obtained by endobronchial brush biopsy of the distal trachea demonstrates upregulation of mRNA for IL-8 (figure 5). Interleukin-8 is a potent cytokine that induces neutrophil chemotaxis (46) which may play a prominent role in neutrophil recruitment to the airway and bronchoalveolar unit. In summary, the inflammatory response to grain dust inhalation is characterized by a neutrophilic alveolitis, activated macrophages that are producing and releasing cytokines, and airway epithelia that produce and probably release interleukin-8. The inflammatory response, as well as the physiologic response, is very similar in individuals exposed to either corn dust extract or LPS; suggesting that LPS plays a prominent role in the pathogenesis of grain dust-induced airway injury.

Endotoxin Concentration and the Inflammatory Response to Grain Dust

Endotoxin largely resides in the high molecular weight fraction (greater than 100 kilodaltons) of corn dust extract (figure 6). Stimulation of THP-1 cells, a myelomonocytic cell line, with the size fractionated solutions of corn dust extract demonstrate that THP-1 cells release TNF-α when stimulated with the high molecular weight fraction of corn dust extract which contains almost all of the endotoxin. In contrast, very little TNF-α is released in response to the other samples of corn dust extract. Treating the high molecular fraction of corn dust extract with either polymyxin B or passing it over an electrostatically charged filter (Zetapor filter; microfiltration products; Meriden, CT) resulted in a significant reduction in the concentration of endotoxin (data not presented), making these high molecular weight solutions of corn dust extract similar in terms of endotoxin concentration to the solution of corn dust extract less than 100 kilodaltons. Using a murine animal model to test these four different samples of grain dust extract, we found that the high molecular weight untreated fraction that contained high concentrations of endotoxin, resulted in inflammation in the lower respiratory tract that was most notable for a high concentration of cells, neutrophils, and TNF-α in the whole lung lavage fluid (figure 7). Interestingly, mice exposed to either the low molecular weight fraction containing minimal amounts of endotoxin or either of the high molecular weight fractions that were treated to reduce the concentration of endotoxin, had substantially less inflammation in the lower respiratory tract than those exposed to the high molecular weight untreated sample of corn dust extract. Restoring the endotoxin concentration in the treated samples resulted in complete restoration of the inflammatory response. These findings suggest that the concentration of endotoxin in corn dust extract strongly influences the inflammatory response to corn dust extract.

LPS Responsiveness and the Inflammatory Response to Grain Dust

If endotoxin is the principal agent in grain dust that causes airway inflammation and airflow obstruction, then modifying the host's ability to respond to endotoxin should substantially alter the host's ability to respond to grain dust. Fortunately, there is an excellent genetic model of LPS hyporesponsiveness that is available for investigation. The C3H/HeJ mouse is a strain of mice that was identified in the early 1960's as being hyporesponsive to endotoxin (50). LPS hyporesponsiveness in C3H/HeJ mice is partially reversible following treatment with trypsin, BCG, γ-IFN, or α-IFN. Inhalation challenge of endotoxin hyporesponsive mice (C3H/HeJ) with corn dust extract results in a negligible inflammatory response in the conducting airways (figure 8). In contrast, endotoxin sensitive mice (C3H/BFEJ) challenged with corn dust extract developed a neutrophilic inflammatory response that was primarily localized around the conducting airways. To further quantify this response, we exposed mice to LPS or to corn dust extract containing various concentrations of endotoxin and examined the inflammatory response by whole lung lavage. Among endotoxin-sensitive mice, a clear dose-response effect was evident with increasing concentrations of endotoxin from either the pure endotoxin solution or the corn dust extract solution and the concentration of neutrophils in the whole lung lavage fluid (figure 9). In contrast, the endotoxin-resistant mice have a negligible inflammatory response to solutions containing the highest concentrations of endotoxin. Similar and consistent findings are observed when we used TNF-α in the whole lung lavage fluid as our measure of lung inflammation (figure 10). Increasing concentrations of endotoxin in the inhalation challenge of either pure endotoxin solution or corn dust extract solution resulted in significant increases in the concentration of TNF-α in the lavage fluid. In contrast, no inflammatory response was observed among

endotoxin-resistant mice even at the highest concentration of inhaled endotoxin.

In the second series of experiments, we investigated whether acquired endotoxin hyporesponsiveness would alter the inflammatory response induced by grain dust by making endotoxin-sensitive mice tolerant to endotoxin. In these experiments, endotoxin-sensitive mice were injected intraperitoneally with increasing daily doses of E. coli LPS (100, 500, 1,000, and 5,000 µg/kg) in a volume of 0.25 ml for 4 consecutive days to induce a state of endotoxin tolerance (51), while control mice were given daily injections of sterile saline. On the 5th day, 18 mice (9 mice from each exposure group) were exposed for 4 h to inhaled LPS at 44 $\mu g/m^3$ and 20 mice (10 from each exposure group) were exposed for 4 h to corn dust extract (endotoxin conc 3.4 $\mu g/m^3$). After exposure, animals were sacrificed and whole lung lavage was performed. Acquired tolerance to endotoxin significantly reduced the inflammatory response to inhaled corn dust (figure 11). Endotoxin-sensitive mice pretreated with intraperitoneal LPS vs. similar mice pretreated with saline demonstrated tolerance to parenterally administered endotoxin with a > 60-fold reduction in serum levels of TNF-α. Moreover, in comparison to control mice, endotoxin-sensitive mice that were made tolerant to endotoxin demonstrated a significantly reduced concentration of lavage cells, neutrophils, and lavage fluid TNF-α when exposed to inhaled corn dust extract (figure 11). Furthermore, each of the measures of inflammation in mice pretreated with endotoxin was reduced by $\geq$ 50% in comparison to control mice.

In conclusion, our studies have shown that grain dust and endotoxin have similar physiologic and biologic effects, that the concentration of endotoxin appears to play an important role in the acute biologic response to grain dust, and genetic and acquired hyporesponsiveness to endotoxin substantially reduces the biologic response to grain dust. In aggregate, these findings strongly suggest that endotoxin is the principal component of grain dust responsible for the development of acute inflammation of the airway as well as the development of airflow obstruction. Since grain dust-induced airway disease appears to be more prominent among those exposed to higher concentrations of endotoxin (21), control of endotoxin concentrations in the workplace may serve to minimize the development of respiratory impairment in grain workers.

References

1. Chan-Yeung M, Enarson DA, Kenney SM. 1992. The impact of grain dust on respiratory health. Am Rev Respir Dis. 145:476-487.

2. Carey P, Hutchson M, Broder I, Mintz S. 1982. Grain elevator workers show work-related pulmonary function changes and dose-effect relationships with dust exposure. Br J Ind Med. 39:330-337.

3. Chan-Yeung M, Schulzer M, MacLean L, Dorken E, Grzybowski S. 1980. Epidemiologic health survey of grain elevator workers in British Columbia. Am Rev Respir Dis. 121:329-338.

4. doPico GA, Reddan W, Anderson S, Flaherty D, Smalley E. 1983. Acute effects of grain dust exposure during a work shift. Am Rev Respir Dis. 128:399-404.

5. Becklake MR, Jodoin G, Lefort L, Rose R, Mandl M, Fraser RG. 1980. A respiratory health study of grain handlers in St. Lawrence River ports. In: Occupational Pulmonary Disease: Focus on Grain Dust and Health. JA Dosman and DJ Cotton, editors. Academic Press, New York. pp. 239:251.

6. doPico GA, Reddan W, Flaherty D, Tsiatis A, Peters ME, Rao P, Rankin J. 1977. Respiratory abnormalities among grain handlers. Am Rev Respir Dis. 115:915-927.

7. Manfreda J, Holford-Strevens V, Cheang M, Warren CPW. 1986. Acute symptoms following exposure to grain dust in farming. Environ Health Perspect. 66:73-80.

8. James AL, Zimmerman MJ, Ee H, Ryan G, Musk AW. 1990. Exposure to grain dust and changes in lung function. Br J Ind Med. 47:466-472.

9. James AL, Cookson WOCM, Buters G, Lewis S, Ryan G, Hockey R, Musk AW. 1986. Symptoms and longitudinal changes in lung function in young seasonal grain handlers. Br J Ind Med. 43:587-591.

10. McCarthy PE, Cockcroft AE, McDermott M. 1985. Lung function after exposure to barley dust. Br J Ind Med 42:106-110.

11. McDuffie HH, Pahwa P, Dosman JA. 1991. Respiratory health status of 3098 Canadian grain workers studied longitudinally. Am J Ind Med. 20:753-762.

12. Chan-Yeung M, Schulzer M, MacLean L, Dorken E, Tan F, Lam S, Enarson D, Grzybowski S. 1981. A follow-up study of the grain elevator workers in the port of Vancouver. Arch Environ Health. 36:75-81.

13. Tabona M, Chan-Yeung M, Enarson D, MacLean L, Dorken E, Schulzer M. 1984. Host factors affecting longitudinal decline in lung spirometry among grain elevator workers. Chest. 85:782-786.

14. Huy T, Schipper KD, Chan-Yeung M, Kennedy SM. 1991. Grain dust and lung function. Am Rev Respir Dis. 144:1314-1321.

15. Dosman JA, Cotton DJ, Graham BL, Li KYR, Froh F, Barnett GD. 1980. Chronic bronchitis and decreased forced expiratory flow rates in lifetime nonsmoking grain workers. Am Rev Respir Dis. 121:11-16.

16. Cotton DJ, Graham BL, Li KYR, Froh F, Barnett GD, Dosman JA. 1983. Effects of grain dust exposure and smoking on respiratory symptoms and lung function. J Occup Med. 25:131-141.

17. Gerrard JW, Mink J, Cheung SC, Tan LKT, Dosman JA. 1979. Nonsmoking grain handlers in Saskatchewan: airways reactivity and allergy status. J Occup Med. 21:342-346.

18. Mink JT, Gerrard JW, Cockcroft DW, Cotton DJ, Dosman JA. 1980. Increased bronchial reactivity to inhaled histamine in nonsmoking grain workers with normal lung function. Chest. 77:28-31.

19. Kennedy SM, Dimich-Ward H, Desjardins A, Kassam A, Sverre V, Chan-Yeung M. 1994. Respiratory health among retired grain elevator workers. Am J Respir Crit Care Med. 150:59-65.

20. Corey P, Hutcheon M, Broder I, Mintz S. 1982. Grain elevator workers show work-related pulmonary function changes and dose-effect relationships with dust exposure. Br J Ind Med. 39:330-337.

21. Schwartz DA, Thorne PS, Yagla SJ, Burmeister LF, Olenchock SA, Watt JL, Quinn TJ. 1995. The role of endotoxin in grain dust-induced lung disease. Am J Respir Crit Care Med. 152:603-608.

22. Donham K, Haglind P, Peterson Y, Rylander R, Belin L. 1989. Environmental and health studies of farm workers in Swedish swine confinement buildings. Br J Ind Med. l 46:31-37.

23. Thelin A, Tegler O, Rylander R. 1984. Lung reactions during poultry handling related to dust and bacterial endotoxin levels. Eur J Respir Dis. 65:266-271.

24. Kennedy SM, Christiani DC, Eisen EA, Wegman DH, Greaves IA, Olenchock SA, Ye T, Lu P. 1987. Cotton dust and endotoxin exposure-response relationships in cotton textile workers. Am Rev Respir Dis. 135:194-200.

25. Castellan RM, Olenchock SA, Hankinson JL, Millner PD, Cocke JB, Bragg K, Perkins HH, Jacobs RR. 1984. Acute bronchoconstriction induced by cotton dust: dose related responses to endotoxin and other dust factors. Ann Intern Med. 101:157-163.

26. Rylander R, Haglind P, Lundholm M. 1985. Endotoxin in cotton dust and respiratory function decrement among cotton workers in an experimental cardroom. Am Rev Respir Dis. 131:209-213.

27. Schwartz DA, Donham KJ, Olenchock SA, Popendorf WJ, Van Fossen DS, Burmeister LF, Merchant JA. 1993. Determinants of longitudinal changes in spirometric function among swine confinement operators and farmers. Am J Respir Crit Care Med. 151:47-53.

28. Chan Yeung M, Wong R, Mac Lean L. 1979. Respiratory abnormalities among grain elevator workers. Chest. 75:461-467.

29. doPico GA, Jacobs S, Flaherty D, Rankin J. 1982. Pulmonary reaction to durum wheat. Chest. 81:55-61.

30. doPico GA, Flaherty D, Bhansali P, Chavaje N. 1982. Grain fever syndrome induced by inhalation of airborne grain dust. J Allergy Clin Immunol. 69:435-443.

31. Gordon T, Balmes J, Fine J, Sheppard D. 1991. Airway edema and obstruction in guinea pigs exposed to inhaled endotoxin. Br J Ind Med. 48:629-635.

32. Olenchock SA, Mull JC, Major PC. 1980. Extracts of airborne grain dusts activate alternative and classical complement pathways. Ann Allergy. 44:23-28.

33. Tobias PS, Mathison J, Mintz D, Lee J-D, Kravchenko V, Kato K, Pugin J, Ulevitch RJ. 1992. Participation of lipopolysaccharide-binding protein in lipopolysaccharide-dependent macrophage activation. Am J Respir Cell. Mol Biol. 7:239-245.

34. Von Essen SG, Robbins RA, Thompson AB, Ertl RF, Linder J, Rennard S. 1988. Mechanisms of neutrophils recruitment to the lung by grain dust exposure. Am Rev Respir Dis. 138:921-927.

35. Lewis DM, Mentnech MS. 1984. Extracts of airborne grain dusts stimulate interleukin-1 (IL-1) production by alveolar macrophages (Abstract). Am Rev Respir Dis. 129:A161.

36. Keller III GE, Lewis DM, Olenchock SA. 1987. Demonstration of inflammatory cell population changes in rat lungs in response to intratracheal instillation of spring wheat dust using lung enzymatic digestion and centrifugal elutriation. Comp Immunol Microbiol Infect Dis. 10:219-226.

37. Von Essen SG, McGranaghan S, Cirian D, O'Neill D, Spurzem JR, Ronnard SI. 1991. Inhalation of grain sorghum dust extract causes respiratory tract inflammation in human volunteers (Abstract). Am Rev Respir Dis. 143:A105.

38. Olenchock SA, Lewis DM, Mull JC. 1986. Composition of extracts of airborne grain dusts: lectins and lymphocyte mitogens. Environ Health Perspect. 66:119-123.

39. Michel O, Duchateau J, Sergysels R. 1989. Effect of inhaled endotoxin on bronchial reactivity in asthmatic and normal subjects. J Appl Physiol. 66:1059-1064.

40. Rylander R, Bake B, Fischer JJ, Helander IM. 1989. Pulmonary function and symptoms after inhalation of endotoxin. Am Rev Respir Dis. 140:981-986.

41. Rylander R, Beijer L. 1987. Inhalation of endotoxin stimulates alveolar macrophage production of platelet activating factor. Am Rev Respir Dis. 135:83-86.

42. Kips JC, Travernier J, Pauwels RA. 1992. Tumor necrosis factor causes bronchial hyperresponsiveness in rats. Am Rev Respir Dis. 145:332-336.

43. Dinarello CA. 1984. Interleukin-1 and the pathogenesis of the acute phase response. N Engl J Med. 311:1413-1418.

44. Sherry B, Cerami A. 1988. Cachectin/tumor necrosis factor exerts endocrine, paracrine, and autocrine control of inflammatory responses. J Cell Biol. 107:1269-1277.

45. Chensue SW, Terebuh PD, Remick DG, Scales WE, Kunkel SL. 1991. In vivo biologic and immunohistochemical analysis of interleukin-1 alpha, beta, and tumor necrosis factor during experimental endotoxemia. Am J Pathol. 138:395-402.

46. Strieter RM, Standiford TJ, Rolfe MW, Kunkel SL. 1993. Interleukin-8. In: Cytokines of the Lung, edited by J. Kelley. New York: Dekker.

47. Clapp WD, Becker S, Quay J, Watt JL, Thorne PS, Frees KL, Zhang X, Koren HS, Lux CR, Schwartz DA. 1994. Grain dust-induced airflow obstruction and inflammation of the lower respiratory tract. Am J Respir Crit Care Med. 150:611-617

48. Schwartz DA, Thorne PS, Jagielo PJ, White GE, Bleuer SA, Frees KL. 1994. Endotoxin responsiveness and grain dust-induced inflammation in the lower respiratory tract. Am J Physiol. 267:L609-L617.

49. Jagielo PJ, Thorne PS, Kern JA, Quinn TJ, Schwartz DA. 1995. The role of endotoxin in grain dust-induced lung inflammation in mice. Am J Physiol (in press).

50. Vogel SN. 1992. The LPS gene. Insights into the genetic and molecular basis of LPS responsiveness and macrophage differentiation. In: Tumor Necrosis Factors: The Molecules and Their Emerging Role in Medicine, edited by Bruce Beutler. Raven Press, Ltd, New York.

51. Coffee KA, Halushka PV, Ashton SH, Tempel GE, Wise WC, Cook JA. 1992. Endotoxin tolerance is associated with altered GTP-binding protein function. J Appl Physiol. 73:1008-1013.

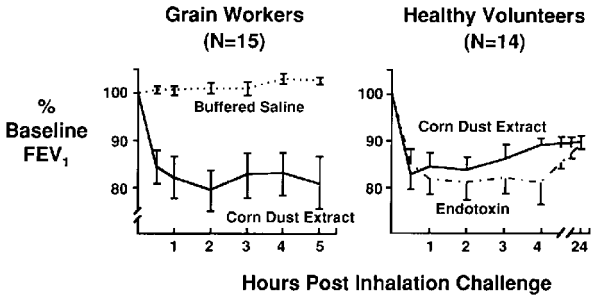

Figure 1. Percentage of baseline forced expiratory volume in one second (FEV1) after inhalation of buffered saline and corn dust extract in grain workers and buffered saline and endotoxin in healthy volunteers.

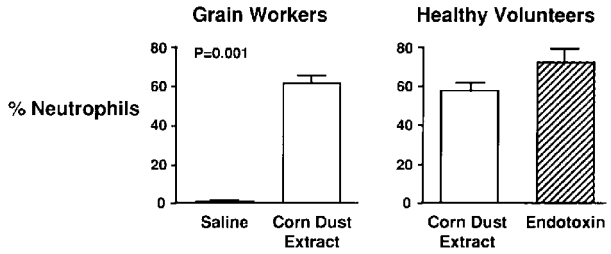

Figure 2. The percentage of neutrophils in the bronchoalveolar lavage fluid four hours after inhalation of saline and corn dust extract in grain workers and corn dust extract and endntoxin in healthy volunteers.

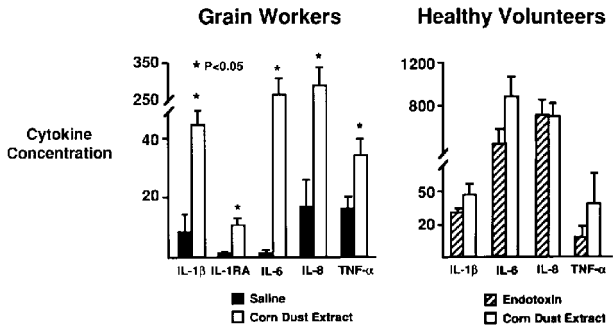

Figure 3. Bronchoalveolar lavage cytokine concentration four hours after inhalation of saline and corn dust extract in grain workers and corn dust extract and endotoxin in healthy volunteers.

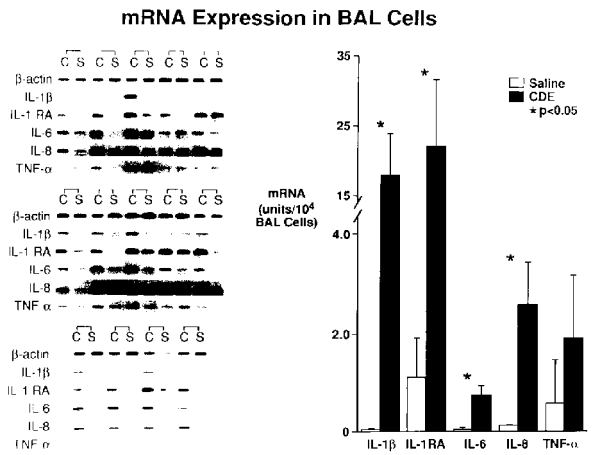

Figure 4. RT-PCR gel and densitometry of bronchoalveolar lavage cell mRNA for IL-1β, IL-1RA, IL-6, IL-8, and TNF-α four hours after inhalation challenge with corn dust extract (c) and saline (s).

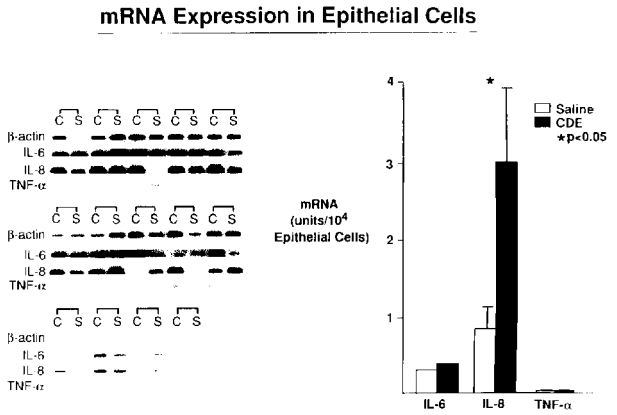

Figure 5. RT-PCR gel and densitometry of airway epithelia mRNA for IL-6, IL-8, and TNF-α four hours after inhalation challenge with corn dust extract (c) or saline (s).

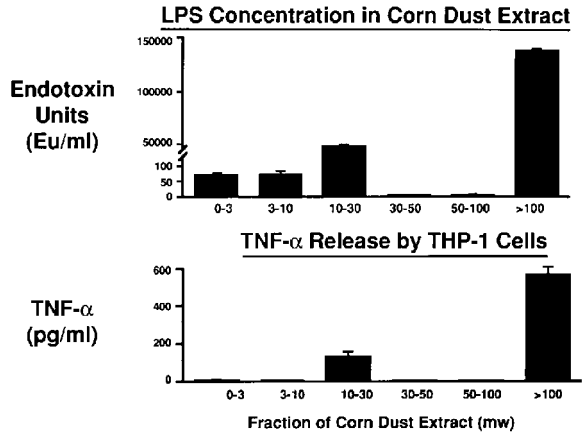

Figure 6. The concentration of endotoxin is shown for each of the molecular weight fractions of corn dust extract in the upper graph. In the lower graph, the concentration of TNF-α in the cell lysate of THP-1 cells is presented for each of the size fractions of corn dust extract solution.

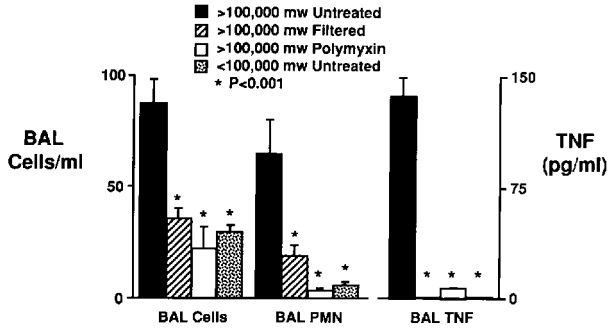

Figure 7. The whole lung lavage concentrations of total cells, neutrophils, and TNF-α are presented following inhalation exposure to four different solutions of corn dust extract (> 100,000 Kd untreated, > 100,000 daltons filtered with an electrostatically charged filter, > 100,000 daltons treated with polymyxin B and < 100,000 daltons untreated).

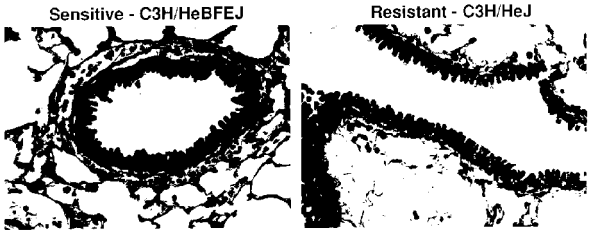

Figure 8. Histologic sections of mouse lungs following inhalation of corn dust extract. The endotoxin-sensitive mice (C3H/HeBFEJ) demonstrates a profound neutrophilc inflammatory response primarily localized to the conducting airway while no inflammatory response is observed in the endotoxin-resistant (C3H/HeJ) mice.

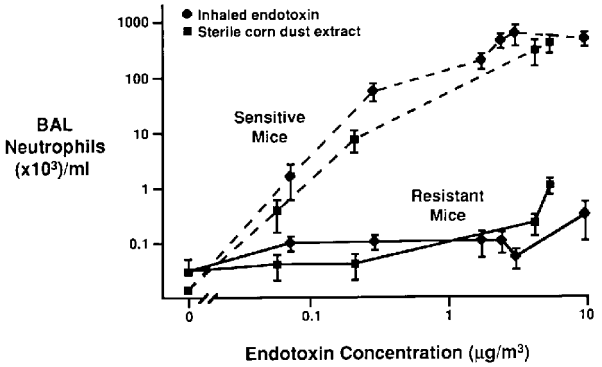

Figure 9. Concentration of total neutrophils in whole lung lavage for endotoxin-resistant (solid line) and endotoxin-sensitive (dash lines) mice in response to increasing doses of inhaled LPS and corn dust extract.

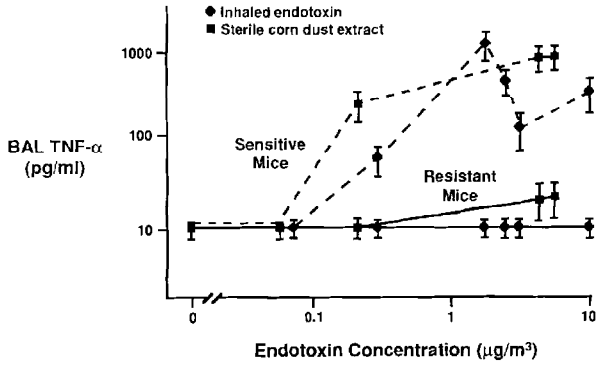

Figure 10. Concentration of total TNF-α in whole lung lavage for endotoxin-resistant (solid lines) and endotoxin-sensitive (dash lines) of mice in response to increasing doses of inhaled LPS and corn dust extract.

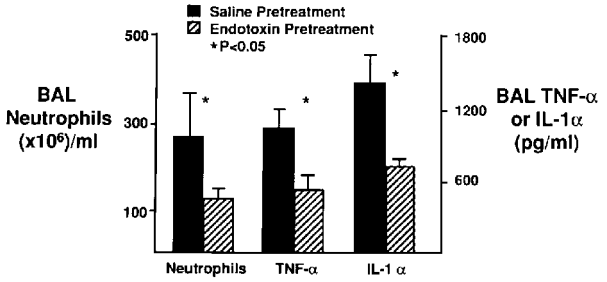

Figure 11. Concentration of whole lung lavage neutrophils, TNF-α, and IL-1β after exposure to inhaled corn dust extract for endotoxin-sensitive mice pretreated with endotoxin (open bars) or saline (solid bars).