

Pulmonary Reactions to Organic Dust Exposures: Development of an Animal Model

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Acute inhalation of organic dusts such as cotton, hay, silage, grain, animal confinement, or compost dust can result in illness characterized by fever, pulmonary inflammation, chest tightness, and airway obstruction. These agricultural materials are complex mixtures of plant, bacterial, and fungal products. Elucidation of the time course of disease onset, the mechanisms of disease progression, and the identity of etiologic agents is essential for effective prevention and treatment. Toward this end, animal models for acute organic dust-induced reactions have been developed and characterized. Information concerning the applicability of various animal models to humans and progress toward elucidation of causative agents and mechanisms of action is presented. — *Environ Health Perspect* 104(Suppl 1):41–53 (1996)

Key words: agricultural dusts, cotton dusts, mechanisms, etiologic agents, byssinosis, organic dust toxic syndrome, endotoxin, exposure system, animal model

Introduction

In 1713, Ramazzini (1) observed that illness resulted from exposure to grain dust. Since then adverse health effects have been described following exposure to numerous organic dusts such as hay (2), grain (3,4), wood (5), and compost (6). Exposure-induced illness has also been reported for workers in swine confinement (7,8), sewage treatment (9), and industrial fermentation facilities (9). Over the years, numerous terms have been used to categorize physiological reactions to various agricultural dust exposures. These myriad syndromes, such as grain fever, organic dust toxic syndrome, toxic pneumonitis, inhalation fever, silo unloaders' disease,

hypersensitivity pneumonitis, farmers' lung, mushroom workers' lung, and bark strippers' disease, have led to considerable confusion (10,11).

Recently, attempts have been made to simplify terminology used to describe reactions to agricultural or organic dust exposure. For clarity, symptoms were divided into responses to either isolated or multiple exposures (12). An isolated exposure to relatively high organic dust concentrations often results in acute flulike symptoms. This illness is called organic dust toxic syndrome (ODTS) and is synonymous with grain fever, silo unloaders' disease, inhalation fever, and toxic pneumonitis. Symptoms begin 4 to 6 hr after exposure and subside after 24 hr. They include fever, chills, headache, myalgia, fatigue, and leukocytosis. Pulmonary symptoms may include chest tightness (2) and reduced forced expiratory volume in 1 sec (FEV₁) (4). Bronchoalveolar lavage samples demonstrate marked infiltration of polymorphonuclear leukocytes (13,14). Chest radiographs may show patchy infiltrates but are often normal (15). Crackles and hypoxemia may also be present (16). Since serum antibodies to the dust in question need not be present,

ODTS is not considered to be a classical immune response (17). In individuals with a history of organic dust exposures, a relatively low-dose exposure can result in hypersensitivity pneumonitis (HP). These individuals have serum antibodies to the organic dust in question and their reactions are typical of a type IV cell-mediated immune response (18). This syndrome is synonymous with farmers' lung, mushroom workers' lung, bark strippers' disease, and allergic alveolitis. Flulike symptoms, such as fever, cough, malaise, and chills occur 4 to 10 hr after exposure. Bronchoalveolar lavage samples are characterized by a lymphocytic infiltration. Pulmonary symptoms include hypoxemia, shortness of breath, compromised diffusion capacity, and rales. Lung infiltrates are commonly apparent on chest X-rays. The acute reactions subside after 18 to 24 hr. However, individuals with hypersensitivity pneumonitis may develop chronic bronchitis as well as granulomatous lesions and diffuse fibrosis, which can be identified radiologically. There may also be pulmonary function evidence of restrictive lung disease (10,12).

Exposure to cotton, flax, or hemp dust is also associated with disease. Historically, illness has been categorized as the response to either single or multiple exposures. Mill fever may occur following the first exposure to a relatively high dose of cotton dust (19,20). This febrile and flulike response is not present after repeated exposures. Chronic exposure can result in byssinosis. This syndrome was first characterized as chest tightness, which is most pronounced on the first day of the work week (Monday accentuation). It begins 2 to 3 hr after exposure starts and increases in severity throughout the workday (21). Schilling (22) introduced a grading system for byssinosis: grade 0—no symptoms; grades 0.5 and 1—chest tightness on occasional or every Monday, respectively; grade 2—chest tightness pronounced on most work days. Although these classic definitions of mill fever and byssinosis are widely used, they do not include the total range of pulmonary responses to cotton dust inhalation (23). For example, cotton textile workers may experience increased airway resistance and decreased FEV₁ upon dust exposure (24). This cross-shift decrease in FEV₁ exhibits Monday accentuation (25). Bronchoconstriction also occurs in volunteers acutely exposed to cotton dust. These study subjects exhibit decreased FEV₁ and

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Abbreviations used: ODTS, organic dust toxic syndrome; FEV₁, forced expiratory volume in 1 sec; HP, hypersensitivity pneumonitis; PMNs, polymorphonuclear leukocytes; BAL, bronchoalveolar lavage; RBCs, red blood cells; PAF, platelet-activating factor; TNF α , tumor necrosis factor- α ; LPS, lipopolysaccharide; INF γ , interferon- γ ; IL-6, interleukin-6; EU, endotoxin units; FMLP, *n*-formyl-methionyl-leucyl-phenylalanine.

increased airway reactivity to methacholine challenge (26,27). Hemp and cotton textile workers exhibit an increase in the number of polymorphonuclear leukocytes (PMNs) in the blood (28) and nasal fluids (20). Likewise, PMNs are elevated in bronchoalveolar lavage samples of byssinotic cotton workers (29). This PMN infiltration can also be demonstrated in volunteers given a single exposure to cotton bract extract (30). There is also a set of pulmonary reactions that occur only after chronic exposure. These include the classical chest tightness (21), a greater than predicted annual decline in FEV₁ (31,32), and bronchitis (33).

A review of the above reactions to an acute exposure to agricultural dusts or cotton dusts reveals that both categories of exposures cause febrile and pulmonary responses that are similar in type and time course. Therefore, it has been proposed that ODS and the acute reactions to cotton dust may occur by similar mechanisms and may be reactions to similar etiologic agents (9,34,35). To fully investigate the reactions to various organic dusts, their dose-dependence and time courses, the mechanisms governing these reactions, and the etiologic agents in these dusts, it is essential to develop animal models that are predictive of the human response. The objective of this paper is to review the development and refinement of such models and to present current information concerning mechanisms and etiology.

Animal Model

Development of an Exposure System

An essential step in the development of an animal model for acute organic dust exposure is the construction of a system for the generation of dust particles of respirable size. Such a system must be able to generate an aerosol of organic dust at controllable and stable levels from bulk samples collected at various work sites.

The first reported cotton dust generator used an ATM sonic sifter to aerosolize particles for inhalation by guinea pigs (36). This generation system was capable of delivering a cotton dust aerosol to an exposure chamber at relatively high concentrations (16–25 mg/m³). The dust generated was respirable, having an aerodynamic equivalent diameter of 3 μm. Although this generator was capable of generating sufficient quantities of dust to induce a pulmonary response in guinea pigs, the generator had to be recharged, i.e., refilled,

with bulk cotton dust collected from textile mills every 15 min to maintain the exposure dose at a reasonably stable level. This limitation was significantly decreased by the development of a Pitt 3 generator (37). This generator used acoustical energy from a loudspeaker delivered through latex rubber dams to a column of air within a plexiglas cylinder to aerosolize bulk cotton dust placed within the cylinder. This system was able to generate cotton dust of respirable size (mass median aerodynamic diameter = 3 μm). The exposure output was relatively stable for 90 min using an initial charge of 30 to 40 g of dust loaded into the cylinder. To conduct inhalation exposures for longer than 1.5 hr, the old bulk material within the cylinder had to be replaced with a new charge (30–40 g) of bulk cotton dust.

Our laboratory was able to substantially improve the performance of the Pitt 3 cotton dust generator. The first improvement was to operate the loudspeaker at the resonant frequency of the generator, i.e., the acrylic cylinder plus the top and bottom rubber diaphragms (38). This modified Pitt 3 generator continued to produce an aerosol of respirable size (mass median diameter = 3.3 μm; count median diameter = 1.6 μm). However, operation at

its resonant frequency (30 Hz) rather than the 60 Hz previously reported (37) improved the stability of the aerosolized dust concentration by 8-fold and allowed operation for 6 hr without recharging with fresh bulk cotton dust. Frazer et al. (39) improved the stability of the dust output further by devising on-line feedback control of the dust concentration in the exposure chamber. This feedback control was effected by placing a miniram (miniature real-time aerosol monitor, GCA model #PDM-3) in the exposure chamber to constantly monitor the dust level in the vicinity of the guinea pigs. The miniram signal was processed by a computer that adjusts the diluent air to maintain a constant particle concentration. A schematic of this exposure system is shown in Figure 1. Using a single charge of 30 g of bulk cotton dust, this improved system can expose guinea pigs to 35 mg/m³ of cotton dust for 6 hr. Over this time, output was stable, i.e., the standard error of the mean for the dust concentration within the exposure chamber was 4%. In addition, particle size varied by less than 6% over this time, and the endotoxin content of the aerosolized dust remained constant. This dust exposure system has since been shown to be equally

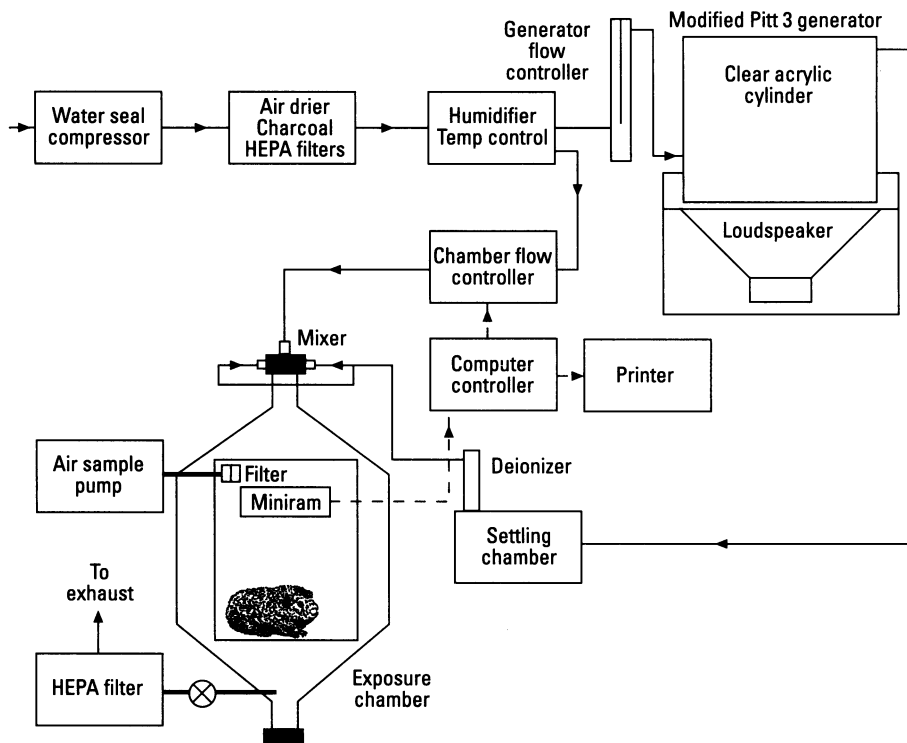


Figure 1. Schematic diagram of an effective generation and exposure system for organic dusts. Data from Frazer et al. (38,39).

effective in generating dust aerosols using bulk samples from various agricultural settings (40,41).

In summary, a system is now available that can generate respirable dust from field samples collected from a variety of occupational sites. The latest version of the generator has the following advantages over previous models: *a*) it is more stable, thus, allowing precise control of dust levels; *b*) operating at the resonant frequency of the generator imparts more energy to the bulk agricultural samples, thus, allowing generation of dust from less dusty samples; *c*) being more stable, the new system can generate lower dust levels that approach levels found in cotton mills; and *d*) since the new system operates for 6 hr on a single dust charge, more exposures can be performed using limited amounts of field samples.

Characterization of Animal Models

Ellakkani et al. (36) were the first to report a time course for breathing pattern changes in guinea pigs exposed to aerosols of cotton dust. They reported a significant decrease in tidal volume and an increase in breathing frequency 3 hr after the start of inhalation of cotton dust at 16 to 25 mg/m³. These breathing pattern changes peaked approximately 18 hr postexposure and were accentuated in the presence of 10% CO₂. Our laboratory later verified this cotton dust-induced change in breathing rate (42). In addition, we reported time courses for the increase in bronchoalveolar lavage (BAL) granulocyte yield, the decrease in BAL yield of alveolar macrophages, and the increase in zymosan-stimulated release of superoxide anion from alveolar macrophages harvested from cotton dust-exposed guinea pigs (42). These data are shown in Figure 2. As with breathing rate, maximal changes in these pulmonary parameters were found 12 to 24 hr postexposure. The time course for the increase in BAL granulocytes and the decrease in BAL macrophages has since been verified (43). The decrease in the number of alveolar macrophages harvested by BAL from cotton dust-exposed guinea pigs may reflect activation of the phagocytes, which caused them to attach more strongly to the lung parenchyma. Support for this possibility is derived from the fact that these cells were activated, producing more superoxide and generating more chemiluminescence (44), and that alveolar macrophage numbers appeared to be elevated in histologic sections from cotton dust-exposed lungs

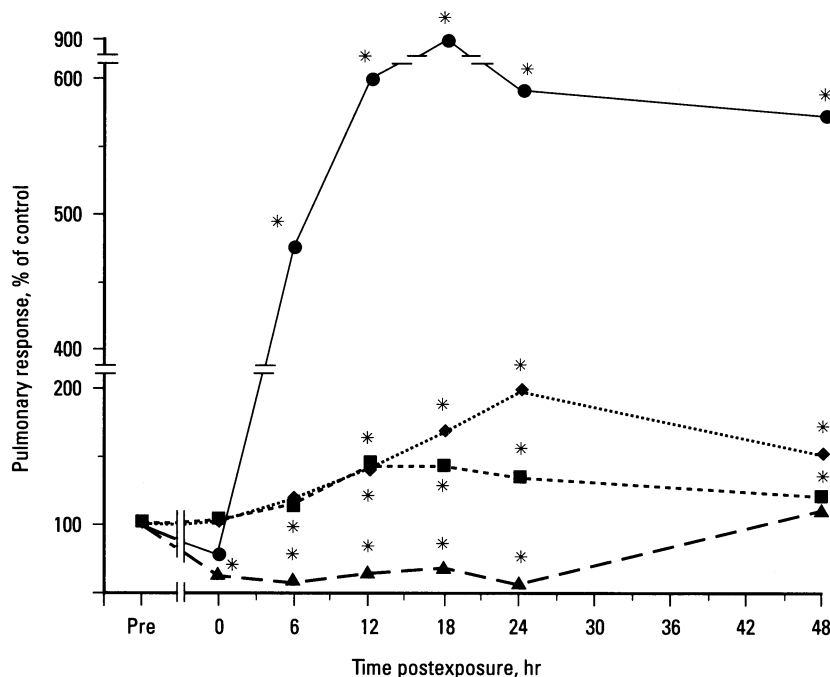


Figure 2. Time course and magnitude of pulmonary responses of guinea pigs to the inhalation of cotton dust. Guinea pigs were exposed to 35 mg/m³ of cotton dust for 2 hr and the following pulmonary responses monitored 0 to 48 hr postexposure: (●) the number of PMNs in BAL samples; (◆) zymosan-stimulated superoxide release from harvested alveolar macrophages; (■) breathing frequency in 10% CO₂; and (▲) number of alveolar macrophages in BAL samples. Asterisk (*) indicates a significant difference from the air controls (*p* < 0.05). Data from Castranova et al. (42).

(Figure 3). Inhalation of cotton dust by guinea pigs also caused disruption of the alveolar air-blood barrier as demonstrated by an increase in lung wet weight/dry weight ratio (45), the appearance of red blood cells (RBCs) in BAL samples (44), and elevated BAL protein levels (46). As with breathing pattern and cellular changes, these parameters peaked approximately 12 to 18 hr postexposure. A febrile response (+1–1.5°C) to cotton dust inhalation has been reported in guinea pigs (47). This response occurred more rapidly than the above reactions, peaking 5 to 8 hr after the onset of exposure. Airway closure (measured as postmortem trapped gas) and increased airway reactivity (measured as sensitivity of excised tracheal smooth muscle to methacholine) also showed a rapid response to cotton dust exposure, being maximal immediately following a 6-hr exposure and decreasing thereafter (45,48). These data are shown in Figure 4. It is of interest that at 18 hr postexposure, when breathing rate and cellular responses are near maximum, airway reactivity was actually lower than normal (48,49).

Ellakkani et al. (50) reported that breathing pattern changes, i.e., increased breathing rate and decreased tidal volume,

occurred in a concentration-dependent manner. Significant changes were first reported after exposure to 6 mg/m³ of cotton dust for 6 hr. Our laboratory extended dose response experiments to include both breathing rate and cellular changes in response to a 6-hr exposure (51). These data indicated that enhanced breathing rate was discernible at 2 mg/m³ of cotton dust, while infiltration of granulocytes into the airspaces and activation of macrophage superoxide production were significant after a dose of 0.4 mg/m³. Not only was the yield of granulocytes in BAL samples a very sensitive indicator of cotton dust exposure, but it also exhibited the largest change from control level of all the parameters tested.

The response of guinea pigs to inhalation of cotton dust depends upon the weight of the animal (52). The increase in breathing frequency was greatest in smaller animals (261 g vs 504 g). This may reflect a greater effective dose of a stimulatory agent per lung surface area in smaller animals. In contrast, granulocyte counts in BAL samples were elevated the most in larger guinea pigs. This may be due to the greater reserve of blood granulocytes that can be recruited in larger animals.

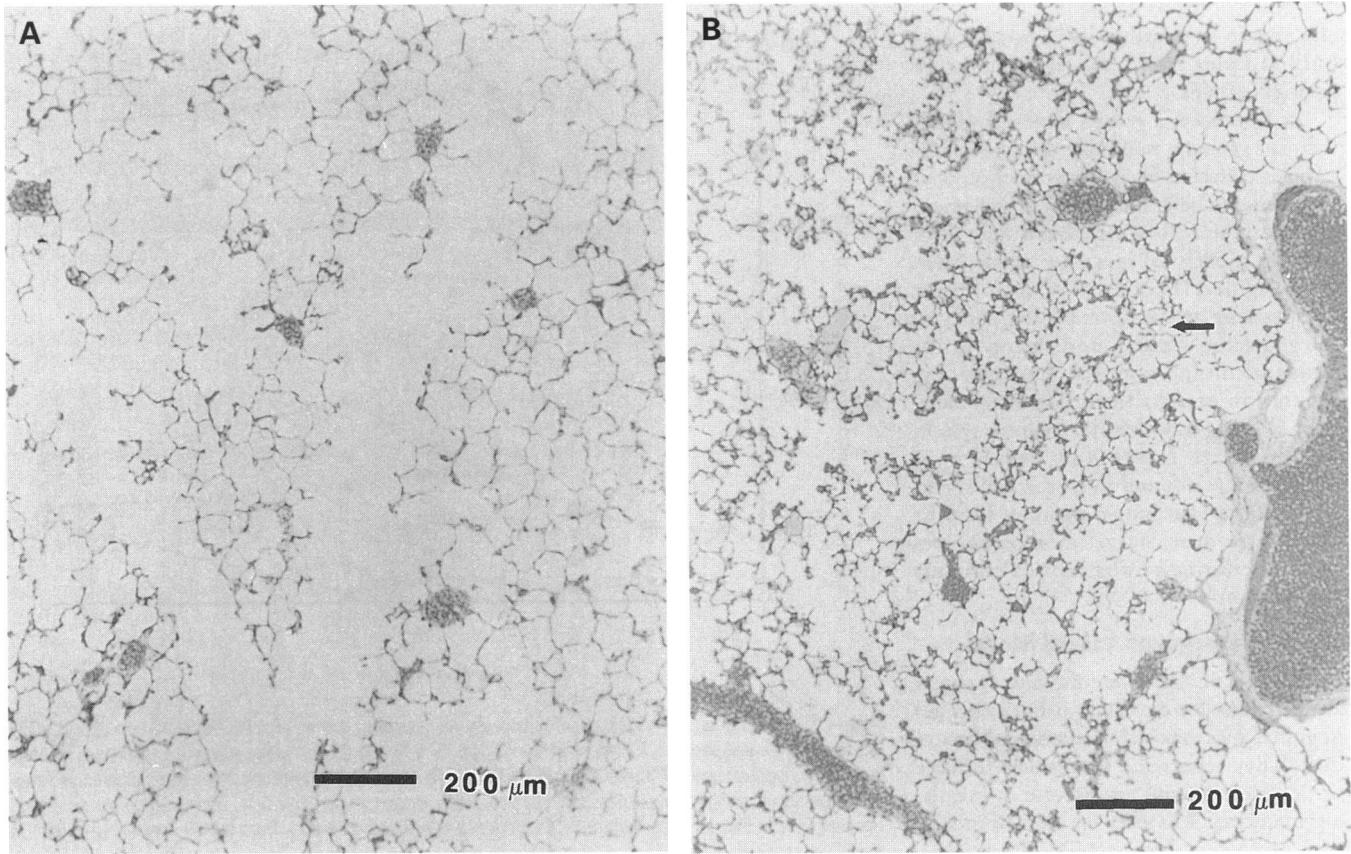
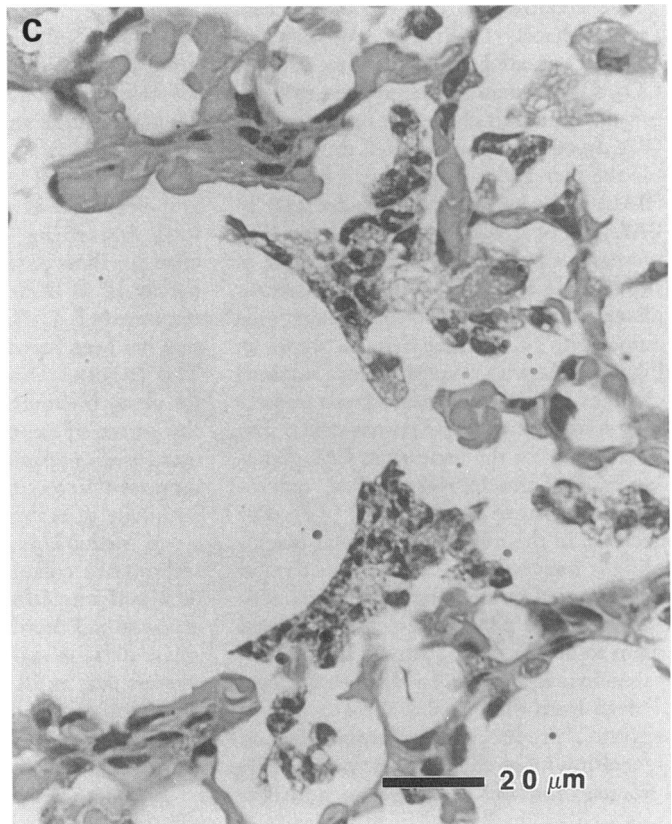


Figure 3. Histological sections of guinea pig lungs. *A*, low-power micrograph of a normal lung; *B*, low-power micrograph of a guinea pig lung 18 hr after a 2-hr exposure to 35 mg/m³ of cotton dust; *C*, high-power micrograph of *B* (note arrow) showing alveolar macrophages and PMNs in the alveolar spaces. Data from Castranova et al. (42).



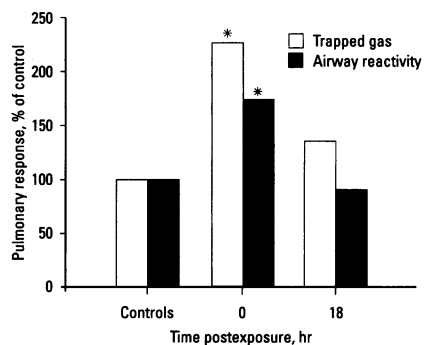


Figure 4. Time course and magnitude of changes in airway closure and reactivity in guinea pig exposed to cotton dust. Airway closure (postmortem trapped gas) was measured in guinea pigs exposed to 20 mg/m³ of cotton dust for 6 hr. Airway reactivity (sensitivity of excised tracheal smooth muscle to methacholine) was measured in guinea pigs exposed to 30 mg/m³ of cotton dust for 6 hr. Asterisk (*) indicates a significant increase from the air control ($p < 0.05$). Data from Frazer et al. (45) and Smith et al. (48).

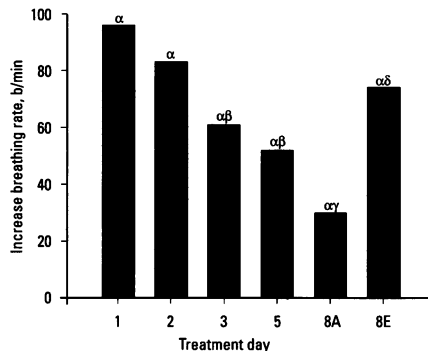


Figure 5. Breathing pattern of guinea pigs in response to repeated cotton dust exposures. Guinea pigs were exposed to 25 mg/m³ of cotton dust for 6 hr, and breathing rate in 10% CO₂ was measured 17 hr postexposure. α Indicates a significant increase from the air control. β Indicates a significant decrease from the day 1 response. γ Indicates a significant decrease from the day 5 response. δ Indicates that the breathing rate after the eighth day of exposure (8E) is significantly greater than the eighth day prior to exposure (8A).

Macrophage activation and airway closure responses to cotton dust exposure did not vary greatly with change in animal weight.

Exercise also affects the magnitude of pulmonary responses to cotton dust inhalation (53). At a given exposure dose, the cellular reaction to cotton dust exposure was increased during exercise (i.e., BAL yield of granulocytes, lymphocytes, and RBCs, as well as superoxide production by alveolar macrophages, was greater in rats that exercised during the inhalation of cotton dust compared to those allowed to be sedentary). This difference may reflect increased deposition or altered distribution or clearance of cotton dust particles in lungs during exercise.

The guinea pig animal model exhibits Monday accentuation of the pulmonary responses to cotton dust exposure. As shown in Figure 5, the elevation in breathing frequency in response to cotton dust was most dramatic on the first day of exposure and decreased with successive exposures during the week. After a weekend to recover, the guinea pig response was again greatest on the first exposure day (Monday) of the next week (54). Similar Monday accentuation was exhibited with the recruitment of granulocytes into the airspaces and the activation of superoxide production by zymosan-stimulated alveolar macrophages (54). The Monday accentuation was demonstrable in guinea pigs even after several weeks of exposure to cotton dust (36).

The chronic reactions of the guinea pig to long-term exposure to cotton dust

(2 mg/m³, 6 hr/day, 5 days/week for 52 weeks) have also been reported. For the first 3 months of exposure, guinea pigs exhibited increased breathing rate and decreased tidal volume, which was most striking after the first exposure of the week (Monday response). Thereafter, the magnitude of breathing-pattern changes increased on days other than Monday until after 6 months of exposure when the severity of these reactions became equal throughout the exposure week (55). After 1 year of cotton dust exposure, total lung capacity and lung weight were elevated compared to age matched controls (55). Histologically, guinea pigs were divided into type 1 and type 2 responders (56). There were no great changes in the pulmonary histology of type 1 responders following a 1-year exposure to cotton dust. In contrast, type 2 responders exhibited thickening of the alveolar septae, type II cell hyperplasia, decreased functional area for gas exchange, hyperplasia of the bronchiolar epithelium, and increased bronchiolar wall thickness.

From the discussion above, it is clear that a guinea pig animal model describing the physiological responses to inhalation of cotton dust is well characterized. Investigations using other species have been much less extensive. We have detailed the pulmonary reactions of rats to cotton dust exposure (42). As with guinea pigs, inhalation of cotton dust increased breathing rate and the BAL yield of granulocytes in rats while decreasing the BAL yield of alveolar macrophages. The magnitude of these

changes, taken as percent of control, were similar in these two species. However, their time course was strikingly different (Figure 6). In rats, breathing rate and granulocyte yield peaked at 0 and 6 hr postexposure, respectively. In contrast, these parameters rose to a maximum 12 to 18 hr postexposure in the guinea pig. The reaction to cotton dust exposure has also been investigated in mice and hamsters. The mouse response was similar to that of rats; i.e., BAL yield of granulocytes increased after inhalation of cotton dust reaching a maximum after 6 hr (57). The recruitment of polymorphonuclear leukocytes into mouse airways has been confirmed histologically. We have also studied cotton dust responses in hamsters in which cotton dust failed to enhance breathing frequency (Castranova et al., unpublished data). However, exposure did increase BAL leukocyte yields. Maximal cell infiltration was noted 6 to 12 hr postexposure, but the magnitude of the cellular response was much smaller in hamsters than in guinea pigs.

It is clear that guinea pigs exhibit numerous pulmonary responses to cotton dust inhalation. We have shown that guinea pigs also respond to a number of agricultural dusts. In response to hay or silage dusts, guinea pigs exhibited increased breathing rate, pulmonary inflammation (measured as increases in BAL polymorphonuclear leukocytes and lymphocytes), alveolar leakage (measured as an increase in BAL RBCs), airway obstruction, and activation of alveolar macrophages (41). The types and time courses of the responses to hay or silage were similar to those for cotton dust (Table 1). Similarly, exposure of guinea pigs to leaf/wood compost dust resulted in elevation of breathing rate, granulocyte infiltration, activation of alveolar macrophages, and airway obstruction, i.e., responses similar to those for cotton dust (Table 1). As for cotton dust exposure, maximal breathing frequency was noted 12 to 18 hr after compost exposure, while maximal airway obstruction was found 0 hr postexposure (40).

In summary, a guinea pig animal model has been developed for organic dust exposures. The physiological reactions of the guinea pig are similar to those reported in humans exposed to organic materials such as cotton or compost (Table 2). In addition, the time courses of pulmonary responses of the guinea pig more closely mimic those of humans than do the rapid responses seen with rats and mice. Therefore, the guinea pig animal model is

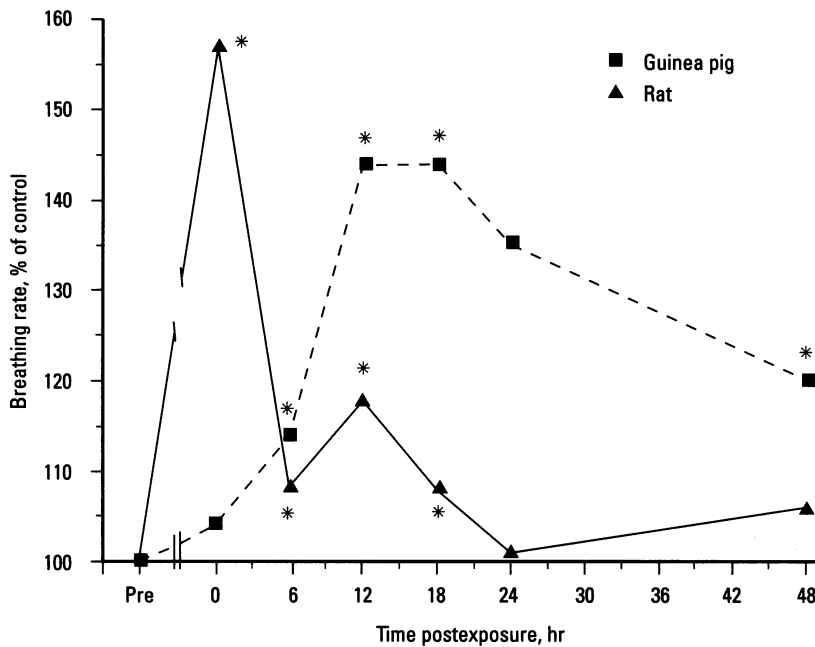


Figure 6. The time course and magnitude of the breathing response in guinea pigs (■) and rats (▲) exposed to 35 mg/m³ of cotton dust for 2 hr. Breathing rate was measured 0 to 48 hr postexposure in the presence of 10% CO₂. Asterisk (*) indicates a significant increase from the air control (*p*<0.05). Data from Castranova et al. (42).

Table 1. Pulmonary responses to cotton and other organic dusts.^a

Pulmonary response	Dust exposure ^b				
	Burnt hay	Chopped hay	Silage	Compost	Cotton
Breathing rate	5	18	4	2	6
PMNs	36	156	198	81	84
Lymphocytes	60	255	84	ND	108
RBCs	45	192	60	ND	60
Superoxide	4	7	4	3	3
Airway obstruction	8	14	2	6	7

ND, not determined. ^aNormalized responses calculated as percent increase over control divided by the exposure dose (40,41). ^bGuinea pigs exposed to dust generated from bulk samples of workplace material that had caused illness in workers.

Table 2. Comparison of human and guinea pig responses to organic dust exposure.

Organic dust	Acute response	References	
		Human	Guinea pig
Cotton	Fever	(19,20)	(47)
	Airway obstruction	(24-26)	(45)
	Airway hyperreactivity	(27)	(48)
	PMNs in BAL	(29,30)	(42)
	AM activation	(30)	(42,44)
	Monday accentuation	(21,25)	(36,54)
	Disease progression	(22)	(55,56)
Compost	Rapid breathing	(6)	(40)
	↑ PMNs	(6)	(40)
	↑ Trapped volume	(6)	(40)

AM, alveolar macrophage.

preferred and can be used to investigate mechanisms of response and to identify etiologic agents related to organic dust exposures.

Elucidation of Mechanisms

As shown in Figure 2, the most marked response of the guinea pig model was the 9-fold increase in lavagable granulocytes in response to organic dust exposure. This infiltration of PMNs was easily demonstrated histologically (Figure 3). There is a striking similarity of the time course of this granulocyte infiltration and changes in breathing rate and macrophage activation (42,58). Therefore, it is possible that cytokines released from inflammatory granulocytes may initiate other pulmonary responses to organic dust exposure. We tested this possibility in guinea pigs exposed to cotton dust (58). Three days before exposure, half the guinea pigs were injected (ip) with cyclophosphamide (200 µg/g bw). Controls received an ip injection of sterile saline. After 3 days, cyclophosphamide-treated guinea pigs were completely depleted of circulating leukocytes due to the action of this agent on hemopoietic cells. Such leukocyte depletion effectively prevented cotton dust-induced recruitment of PMNs into the lungs, as indicated by PMN yield in BAL samples (Table 3). In the absence of PMN recruitment, the breathing rate and macrophage activation responses to cotton dust were decreased by 75 and 100%, respectively. Data from our laboratory also indicate that PMNs isolated from cotton dust-exposed guinea pigs directly induced constriction of isolated tracheal smooth muscle (59). Therefore, PMNs may play a role in the cross-shift declines in FEV₁ reported in cotton textile workers.

Since the pulmonary response to cotton dust exposure is at least partially dependent upon PMN infiltration into the airspaces, efforts have been made to determine how PMNs are recruited into the lungs and activated. Alveolar macrophages exposed to cotton dust release metabolites of arachidonic acid (60). A significant portion of these products are leukotrienes (61). Leukotriene B₄ is a potent chemoattractant for PMNs while leukotrienes C₄ and D₄ cause airway constriction. Therefore, leukotrienes may play a role in the response to organic dust.

We have reported that cotton dust can stimulate the release of platelet-activating factor (PAF) from alveolar macrophages *in vitro* (62). Inhalation of cotton dust has

Table 3. Effect of leukocyte depletion on pulmonary responses to cotton dust.

Group	Pulmonary response		
	PMN ^a	Breathing rate ^b	Superoxide production ^c
Cotton-saline	↑ 1093%	↑ 100%	↑ 52%
Cotton-cyclophosphamide	↑ 13%	↑ 25%	↑ 0%

Values given are percent increases above the respective air control group 12 to 18 hr postexposure. Guinea pigs were treated ip with cyclophosphamide (200 µg/g bw) 3 days prior to inhalation exposure to cotton dust (35 mg/m³ for 2 hr). Modified from Castranova et al. (58). ^aPMNs are polymorphonuclear leukocytes obtained by bronchoalveolar lavage. ^bBreathing rate measure in 10% CO₂. ^cZymosan-stimulated superoxide release from harvested alveolar macrophages.

also been shown to stimulate the production of platelet-activating factor from alveolar macrophages, with maximum release occurring 2 hr postexposure (63,64). PAF is a potent chemoattractant for PMNs (65). In addition, PAF is a direct stimulator of the secretory activity of PMNs while, like cotton dust in the guinea pig model, it potentiates zymosan-stimulated superoxide release and chemiluminescence from alveolar macrophages (62,66). PAF is also a bronchoconstrictor (67) and has been implicated as a mediator of decreases in FEV₁ (66). Therefore, it has been proposed that PAF plays a role in the pulmonary response to cotton dust. This hypothesis has been questioned by Gordon et al. (68) who reported that pretreatment of guinea pigs with a PAF antagonist did not block pulmonary reactions to inhaled endotoxin. However, Burrell et al. (69) reported that the PAF antagonist RP48740 was an effective inhibitor of PMN influx and capillary leakage in guinea pigs treated with endotoxin.

Ryan and Karol (43) reported that cotton dust inhalation by guinea pigs resulted in a significant elevation of tumor necrosis factor- α (TNF α) levels in BAL samples 3 hr after exposure onset. A similar response has also been reported in cotton dust-exposed mice (57). This TNF α appears to be secreted from alveolar macrophages. Indeed, both resting release and lipopolysaccharide (LPS)-stimulated secretion of TNF α was elevated from alveolar macrophages harvested from guinea pigs either immediately following or 1.5 hr after a 6-hr exposure to 33 mg/m³ of cotton dust (43). Additionally, BAL and serum levels of interferon- γ (INF γ) and interleukin-6 (IL-6) were increased immediately following a 3-hr exposure of mice to 58 mg/m³ of cotton dust (70). TNF- α , INF γ , and IL-6 reportedly play a role in pulmonary inflammatory reactions (71,72). Therefore, they may act as mediators of the pulmonary response to cotton dust inhalation. Indeed, Shvedova et al. (46) reported that

ip injection of mice with anti-TNF α anti-serum at 40, 15, and 1 hr prior to cotton dust exposure reduced pulmonary inflammation (number of BAL cells) by 75%, leakage at the alveolar air-blood barrier (BAL protein) by 100%, and BAL levels of TNF α and IL-6 by 40%.

In summary, inhalation of cotton dust stimulates alveolar macrophages to produce mediators which are chemoattractants for PMNs. The recruitment of PMNs seems to be a critical step in many of the pulmonary reactions to exposure. In addition, TNF α appears to be an important mediator of cotton dust-induced pulmonary inflammation.

Identification of Etiologic Agents

Endotoxin is a lipopolysaccharide-protein complex derived from the cell walls of Gram-negative bacteria. LPS is the lipopolysaccharide portion of endotoxin and lipid A is its biologically active component (73). Endotoxin has been shown to cause airway obstruction and recruitment of PMNs into the airways of exposed human subjects (74,75). Since bacterial contamination of organic dusts is common, endotoxin has been considered a prime etiologic agent in numerous occupational settings (6,8,76-79). Castellan et al. (26,80) have shown a strong correlation between the endotoxin content of cotton dust and bronchoconstriction in human volunteers with a threshold at 90 EU/m³ of endotoxin. Their results show that decreases in FEV₁ related more strongly to endotoxin content than to the mass exposure level of total dust. Indeed, washing cotton to lower the endotoxin content caused cotton dust to be a less potent inducer of airway obstruction (81). A similar correlation has also been noted in an epidemiological study of the incidence of byssinosis in cotton textile workers (82).

Animal models also exhibit a strong correlation between the endotoxin level and pulmonary responses to cotton dust inhalation. Karol et al. (83) reported that the ability of various cotton dusts to increase

breathing frequency was directly related both to their capacity to decrease FEV₁ in human volunteers and to their endotoxin content. Using cotton grown in dry versus wet regions of the country (California vs Mississippi, respectively), our lab has demonstrated a linear relationship between breathing rate or BAL cell yield and the endotoxin content of the cotton dust inhaled by guinea pigs (84). We have also shown that a 73% reduction of the endotoxin associated with bulk cotton (due to heating at 250°C) resulted in a proportional reduction (60%) in the cellular inflammatory response of guinea pigs (44). The dependence of changes in breathing pattern and the Monday accentuation of this response on the endotoxin content of inhaled dust was also demonstrated using cellulose dust (85,86). Untreated cellulose did not alter the breathing frequency of guinea pigs; however, cellulose treated with Gram-negative bacteria, i.e., the source of endotoxin, mimicked the response to cotton dust. The response of the guinea pig model to organic dusts other than cotton dust has also been shown to be related to the relatively high endotoxin levels in these materials (40,41).

To further investigate the role of endotoxin or other chemicals in the pulmonary response of the guinea pig animal model to organic dusts, a system must be devised to generate a liquid aerosol of the chemicals in question. Such a generator is shown in Figure 7 (87). In this system, a solution of endotoxin can be aerosolized using an ultrasonic nebulizer, large droplets can be removed in a settling chamber, and respirable size particles can be mixed with diluent air before it enters the exposure chamber. The exposure concentration can be monitored continuously using a miniram to provide computerized feedback control of diluent air, thus stabilizing the delivered dose. Using such a generation system, our laboratory has demonstrated that breathing rate and BAL cell yield of guinea pigs increased in a dose-dependent fashion in response to inhaled endotoxin (88). As with cotton dust exposure, PMN recruitment into the airspaces was the most sensitive response to endotoxin inhalation, peaking at 1 µg/ml of endotoxin, while maximal breathing frequency occurred at 1000 µg/ml of endotoxin. The rapid breathing rate and PMN recruitment in response to endotoxin inhalation has also been reported by others (89-91). In addition, as with cotton dust exposure, inhalation of endotoxin resulted in fever (92),

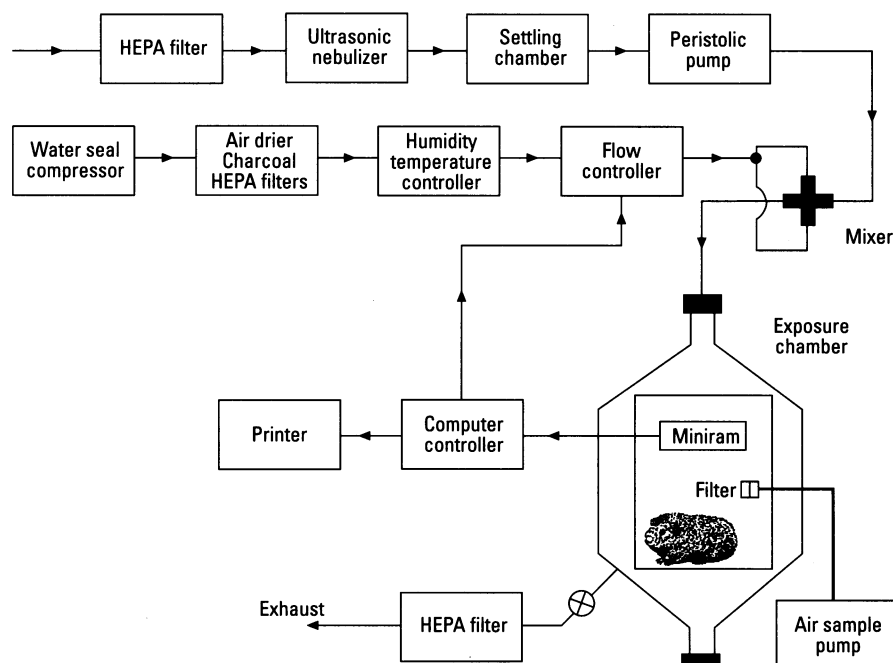


Figure 7. Schematic diagram of an effective generation and exposure system for chemicals in solution. Data from Frazer et al. (87).

protein leakage (68), edema (93), and a decrease in airway conductance (68,93) in guinea pigs. Endotoxin exposure also induced the release of mediators such as TNF α (57,94) and PAF (95) in a manner similar to cotton dust exposure.

The data above support the conclusion that endotoxin is a major etiologic agent in cotton and other organic dusts. Recent data suggest that it may be the only important agent, since endotoxin-resistant mice did not exhibit a pulmonary response following inhalation of cotton dust (57). However, such a conclusion appears premature, since the mouse model was also insensitive to another bacterial product, i.e., the chemotactic peptide *n*-formyl-methionyl-leucyl-phenylalanine (FMLP) (96). Indeed, cotton extracts have been reported to contain an unidentified chemoattractant for neutrophils, that is distinct from endotoxin and has a molecular weight comparable to FMLP (97). An etiologic agent associated with organic dust, in addition to endotoxin, is supported by evidence that grain dust extracts remained chemotactic to neutrophils even after removal of endotoxin (98). Similarly, cotton bract extracts, purified by column chromatography to remove endotoxin, still caused bronchoconstriction in human volunteers (99). At relatively high doses, this cotton bract extract caused a significant increase in breathing rate and cellular

inflammation in guinea pigs (100); however, it should be noted that this cellular and breathing rate response was smaller than that seen with cotton dust exposure. Lastly, cotton dust extract caused contraction of isolated tracheal smooth muscle, whereas endotoxin did not exhibit this contractile action (101,102).

The data above suggest that while endotoxin is an important etiologic agent in organic dust, it may not be the only agent. Recently, our laboratory has reported the presence of FMLP, in both the reduced and oxidized form, in cotton dust extracts (103). Both forms of FMLP exhibited chemotactic activity for neutrophils and activated the production of inflammatory products by PMNs (59,104). Unlike endotoxin, FMLP was also a direct constrictor of tracheal smooth muscle (59). Therefore, since bacteria are common contaminants of organic dusts, FMLP may play a role in the pulmonary inflammation and airway obstruction characteristic of

acute illness associated with exposures in cotton mills and agricultural settings. To determine the role of the chemotactic peptide (FMLP) in these syndromes, inhalation studies were conducted using the guinea pig animal model. As shown in Table 4, inhalation of a liquid aerosol of FMLP (1 mg/m³ for 4 hr) increased breathing frequency, induced pulmonary inflammation (increased BAL yield of PMNs and lymphocytes), caused leakage at the alveolar air-blood barrier (increased BAL yield of RBCs), and activated alveolar macrophages (increased zymosan-stimulated superoxide) (87). These responses were qualitatively similar to those in response to inhalation of cotton or agricultural dusts. However, on a delivered FMLP basis, this agent cannot account for the magnitude of the pulmonary response to organic dust exposure. That is, from the exposure levels given in Table 4, the delivered dose of FMLP was calculated to be approximately 6 times greater in the liquid aerosol exposure than in the cotton dust exposure. Therefore, FMLP may contribute to the pulmonary response to organic dust inhalation, but it is not the sole etiologic agent.

Bell and Stipanovic (105) reported the presence of tannins in cotton dust and suggested that they may play a role in the pulmonary response to cotton dust. As with *in vitro* treatment with cotton dust extract, tannins induced the release of chemotactic factors, metabolites of arachidonic acid, and interleukin-1 from alveolar macrophages (106); however, tannins failed to contract smooth muscle or induce histamine release from mast cells (106). *In vivo*, tannins have been shown to recruit PMNs into the air spaces of hamsters and rats (107). However, most human data are negative concerning the ability of tannins to decrease FEV₁ (107). In the face of these conflicting conclusions, the role of tannins in the pulmonary reaction to cotton dust was tested using the guinea pig animal model. Rylander (108) reported that chemical removal of tannins from cotton dust reduced its ability to recruit

Table 4. Response of guinea pigs to the inhalation of FMLP.^a

Exposure	Breathing rate	PMNs	Lymphocytes	RBCs	Superoxide
FMLP ^b	42%	820%	240%	100%	21%
Cotton dust ^c	70%	974%	1253%	696%	35%

^aMaximal percent increase above control for breathing rate, BAL yield of polymorphonuclear leukocytes, lymphocytes, RBCs, and zymosan-stimulated superoxide release from alveolar macrophages. ^bGuinea pigs were exposed to 1 mg/m³ of FMLP for 4 hr (87). ^cGuinea pigs were exposed to 11.6 mg/m³ of cotton dust for 6 hr (41); cotton dust contains 9.2 mg FMLP/g dust (59).

PMNs into the airways by only 30%. Similarly, removal of tannins from cotton dust did not alter its ability to increase breathing frequency in exposed guinea pigs (109). Inhalation of cellulose powder treated with tannin also failed to alter the breathing pattern of guinea pigs and resulted in only mild recruitment of PMNs into the airspaces (109). These results indicate that tannins do not independently play a major role in acute byssinosis.

Organic dust exposures are often characterized by high levels of viable fungi and fungal spores (6). β -1,3 glucan, a component of fungal cell walls, has been reported to exhibit biological activity toward alveolar macrophages (110). This fungal product has been found in cotton dust (111) and in agricultural dust (112); however, inhalation studies with guinea pigs indicate that β -glucan did not recruit PMNs into the airspaces (113,114). In striking contrast to endotoxin, inhalation of β -glucan failed to alter breathing rate, induce inflammation (BAL yield of leukocytes), cause leakage at the alveolar air-blood barrier (BAL yield of RBCs), or activate alveolar macrophages (chemiluminescence) in mice (Castranova et al., unpublished results). Therefore, data fail to support β -glucan as a major independent etiologic agent in organic dusts.

In summary, animal models have made significant contributions to the identification

of etiologic agents in cotton and other organic dusts. Endotoxin and, to a lesser degree, FMLP are bacterial products that may be important in the etiology of the acute response to agricultural dusts. In contrast, tannins and β -glucan appear to be less critical biologically active agents, although they may play roles in modifying pulmonary responses to the endotoxin in organic dusts. Indeed, recent data from our laboratory indicate that the pulmonary response of guinea pigs, rats, and mice to inhaled endotoxin was modulated by aerosolized FMLP, i.e., breathing rate and the recruitment of PMNs into the air spaces were lower in the presence of endotoxin plus FMLP than with endotoxin alone (115).

Conclusion

Animal models have been developed that mimic the febrile and pulmonary reactions of humans to organic dust inhalation. These models have been useful in identifying endotoxin as a major etiologic agent common to these materials. The guinea pig animal model has been used to test the feasibility of proposed prevention measures that remove endotoxin from bulk cotton by acid/base treatment, heating, or washing. Such studies will no doubt continue. Studies with animal models have also been instrumental in the growing scientific

awareness that acute reactions to cotton dust, compost, grain dust, and other organic materials have a common basis with regard to etiologic agents and mechanisms of disease development.

Although much has been learned, several research gaps remain. First, more mechanistic information with regard to cellular interactions and cytokine control is necessary. Such information is required if effective treatments are to be developed. For example, the acute reaction to inhalation of organic dusts is pulmonary inflammation. Would anti-inflammatory drugs be useful or would they inhibit clearance and prolong adverse effects? Second, more information is required concerning chronic responses of the animal models to organic dust inhalation. Do acute reactions progress to chronic disease such as hypersensitivity pneumonitis? Can progression to chronic disease be inhibited? Are current animal systems useful in modeling chronic human disease? There is also the question of mixed exposures common in the agricultural setting. Will the inflammatory response to organic dust modify pulmonary reactions to inhalation of chemical agents such as pesticides, solvents, or fuels? It is clear that animal models will play an important role in resolving these questions.

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