Intranasal organic dust exposure-induced airway adaptation response marked by persistent lung inflammation and pathology in mice

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Poole JA, Wyatt TA, Oldenburg PJ, Elliott MK, West WW, Sisson JH, Von Essen SG, Romberger DJ. Intranasal organic dust exposure-induced airway adaptation response marked by persistent lung inflammation and pathology in mice. Am J Physiol Lung Cell Mol Physiol 296:L1085–L1095, 2009. First published April 24, 2009; doi:10.1152/ajplung.90622.2008.—Organic dust exposure in agricultural environments results in an inflammatory response that attenuates over time, but repetitive exposures can result in chronic respiratory disease. Animal models to study these mechanisms are limited. This study investigated the effects of single vs. repetitive dust-induced airway inflammation in mice by intranasal exposure method. Mice were exposed to swine facility dust extract (DE) or saline once and once daily for 1 and 2 wk. Dust exposure resulted in increased bronchoalveolar lavage fluid neutrophils and macrophages after single and repetitive exposures. Lavage fluid TNFα, IL-6, keratinocyte chemotactant, and macrophage inflammatory protein-2 were significantly increased after single and repetitive dust exposures, but were dampened in 2-wk dust-exposed mice compared with single exposure. Dust exposure induced PKCε and ε activity in isolated macrophages derived from monocytes/macrophages, and that this response is, in part, regulated through PKC activity, particularly the PKCe isoforms (31, 32). In addition, repeat dust exposure results in an adaptation/tolerance response in human monocytes/macrophages. However, the mechanisms underlying this apparent modulated inflammatory response to single vs. repetitive dust exposures in vivo may be not clear.

In this study, we hypothesized that single vs. repetitive organic dust exposure (DE) from swine confinement facilities intranasally delivered would result in significant airway and lung parenchymal inflammation in mice, and that these inflammatory responses would parallel activation of PKCe activity. We also hypothesized that the alveolar macrophage antigen presenting cell; phagocytosis; cytokines; aggregate; airway hyperresponsiveness

Agricultural workers, particularly swine farmers, exhibit a high prevalence of airway disease including chronic bronchitis, chronic obstructive pulmonary disease, and exacerbation of asthma, which is thought to be due, in part, to chronic organic dust exposure (37). Initial exposure to an organic dust environment induces an intense airway inflammatory response marked by fevers, bronchial hyperresponsiveness, and increases in bronchoalveolar lavage (BAL) fluid neutrophils, macrophages, and proinflammatory mediators including TNFα, IL-6, and IL-8 (CXCL8) (11, 41). Repetitively exposed swine farmers also experience an increase in inflammatory cells and cytokines, but this response is diminished compared with acutely exposed naive subjects (28), suggestive of an adaptation response (37). However, despite an apparent adaptation response, persons repetitively exposed to organic dust experience a high prevalence of chronic respiratory disease and a progressive loss of lung function (34).

Animal models to study the mechanisms that regulate the immune inflammatory response to single vs. repetitive organic dust exposure may be relevant for a better understanding of the respiratory symptoms of organic dust-induced diseases and the well-recognized adaptation-like response. We have previously found, in vitro, that single exposure to this dust induces significant secretion of proinflammatory mediators (TNFα, IL-6, IL-8) in human epithelial cells and monocytes/macrophages, and that this response is, in part, regulated through PKC activity, particularly the PKCα and PKCe isoforms (31, 32). In addition, repeat dust exposure results in an adaptation/tolerance response in human monocytes (31), and repetitive dust exposure impairs the differentiation and function of macrophages derived from monocytes (30). However, the mechanisms underlying this apparent modulated inflammatory response to single vs. repetitive dust exposures in vivo are not clear.

Animal models to investigate these observations are limited. One potential model is placing rodents in hanging cages within the swine barn. This attractive but difficult model has demonstrated airway hyperresponsiveness (AHR) after 1 day of exposure and an AHR adaptation-like response after 1 mo. However, mechanistic studies would be limited as this model fails to demonstrate consistent increases in lung lavage inflammatory cytokines, and lung parenchymal changes, even after 1 mo, are subtle (8, 9). An alternative model proposed in this study is intranasal inhalation of the organic dust. Intranasal administration of other agents, including, but not limited to, ultrafine particles, biomass particles, bacterial components, microbial superantigens, and allergens, are becoming increasingly utilized to investigate mechanisms of exaggerated airway inflammation in vivo (10, 15, 22, 24, 26).

In this study, we hypothesized that single vs. repetitive organic dust exposure (DE) from swine confinement facilities intranasally delivered would result in significant airway and lung parenchymal inflammation in mice, and that these inflammatory responses would parallel activation of PKCα and PKCe. We also hypothesized that the alveolar macrophage (aMφ) would be impaired in mice repetitively exposed to the dust. To test these hypotheses, we compared lavage fluid inflammatory cells and cytokines, PKC isoform activity in isolated tracheal epithelial cells, ex vivo alveolar macrophage responses, lung pathology, and airway hyperresponsiveness to single and repetitive dust exposure in mice.
frozen at -70°C. The supernatant from the first milliliter of BAL fluid recovered was centrifuged at 2500 × g for 10 min, and the supernatant was collected for analysis. The BAL fluid was centrifuged again and the final supernatant was analyzed. The analysis of the BAL fluid was performed on a standard published procedure (32).

In prior experiments, optimal DE concentration was determined by randomizing mice to a treatment group: no handling, PBS (control), or DE control (Invitrogen, Carlsbad, CA). The treatment groups were: concentrations of 1%, 5%, 12.5%, 19%, and 25% DE once daily for 2 wk. The lung tissue was harvested from each animal and the tissue was processed for whole lung lavage. The lungs were pooled due to limited cell numbers: eight mice per group and each group was pooled due to limited cell numbers: eight mice per group.

Animal model and exposure. Male C57BL/6 mice (6–8 wk old) obtained from Charles River (Wilmington, MA) were housed in group cages and fed commercial rodent chow and water ad libitum for a 1-wk acclimation. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Omaha Veterans Affairs Medical Center and the University of Nebraska Medical Center.

In prior experiments, optimal DE concentration was determined by randomizing mice to a treatment group: no handling, PBS control (Invitrogen, Carlsbad, CA), or DE concentrations of 1%, 5%, 12.5%, 19%, and 25% DE once daily for 2 wk. In subsequent time course studies shown here, the optimal concentration of 12.5% DE or saline control was utilized. Mice assigned to an inhalation group received saline control or DE once daily for 1 day, 1 wk, and 2 wk with weekends excluded. To conduct a semiquantitative evaluation of the dose-response inflammatory changes observed after 2 wk of once-daily DE exposure, concentrations of 1%, 5%, 12.5%, and 25% saline (control) were utilized. The procedure for nasal inhalation was performed on an established model using cigarette smoke extract (13, 25).

Briefly, mice were anesthetized by isoflurane inhalation before inhaling the smoke (400 mg/m³) for 2 h. After whole lung lavage, lungs were harvested from each treatment group and each group was pooled due to limited cell numbers: eight mice per group and each group was pooled due to limited cell numbers: eight mice per group.
gen), and 80 U/ml amphotericin B (Invitrogen). Adherent aMΦ were then collected, counted, and assurred for viability by trypan blue exclusion method. For ex vivo cytokine responsiveness studies, 2 × 10^5 cells/ml were stimulated with DE (1%) for 5 h, and cell-free supernatants were subsequently harvested and stored at −20°C until assayed for cytokine secretion by sandwich ELISA, as described above. Cytokine secretion is reported as concentration (in picograms per milliliter) per 2 × 10^6 viable cells, as determined on completion of the experimental protocol by means of the trypan blue exclusion method.

The phagocytic ability of the aMΦ from the 2-wk dust-treated mouse group and saline control group was assessed by flow cytometry utilizing previously published methods (1, 30). Briefly, Saccharomyces cerevisiae zymosan A BioParticles (Molecular Probes, Eugene, OR) conjugated to FITC were opsonized with opsonizing reagent (IgG) for 45 min. After being washed, aMΦ at 5 × 10^6 cells/ml were incubated with FITC-labeled zymosan (Molecular Probes) at 5 × 10^6 particles/ml (1:10 ratio) for 0 and 60 min in the presence of 10% murine serum. Cells were fixed with 1% paraformaldehyde and analyzed on the same day of particle exposure by flow cytometry. Flow cytometric analyses were performed with the FACSCalibur dual-laser cytometer (Becton-Dickinson, Lincoln Park, NJ). Particle uptake was identified as a rightward shift in fluorescence on histogram analysis, and phagocytic ability was determined by assessing the average mean fluorescence intensity (MFI) from the proportion of cells in the zymosan-exposed population at 60 min compared with cells exposed for 0 min (expressed as fold change in MFI).

Immunohistochemistry. Formalin-fixed, paraffin-embedded sections of 4- to 5-μm-thick tissue were deparaffinized through two exchanges of xylene and rehydrated using a graded series of alcohol washes (100%, 95%, 80%, 50% ethanol) and rinsed twice in PBS. Antigen unmasking was performed using the heat-induced epitope retrieval method (42). Slides were immersed in preheated antigen retrieval solution (DIVA Decloaker solution; Biocare Medical, Concord, CA) and steamed for 30 min at 95°C in a vegetable steamer. After being cooled, slides were rinsed with TBST washing buffer (Tris-based with sodium chloride and Tween 20). Endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 15 min. After being washed, slides were blocked for 30 min in a humidiﬁer chamber in 10% normal goat serum or 10% normal rabbit serum (Vector, Burlingame, CA) before application of primary antibodies. Slides were incubated with the following primary antibodies for 1 h in a humidity chamber: rabbit anti-CD3 (Pan-T cell marker, dilution 1:300; Dako, Carpinteria, CA), rat anti-CD45R/B220 (Pan-B cell marker, clone RA3-6B2, dilution 1:200; BD Pharmingen, San Jose, CA), anti-Mac-3 (mononuclear phagocytes, clone M3/84, dilution 1:50; BD Pharmingen).

After being washed, slides were incubated with the appropriate biotinylated goat-anti-rabbit IgG (dilution 1:500) or biotinylated anti-rat IgG mouse-absorbed (dilution 1:500) secondary antibody in a humidity chamber. Biotinylated antibodies were purchased from Vector. After 1 h, slides were rinsed, and primary antibody binding was detected using the avidin-biotin-immunoperoxidase method (Vectastain Elite ABC ready-to-use kit, Vector). Chromogen substrate (IMMPPACT DAB, Vector) developer was used, and slides were counterstained with 1% Meyer’s hematoxylin. Slides were dehydrated through a series of ethanol and fixed with xylene.

Invasive pulmonary function measurement. The standard model to measure respiratory mechanics is by the linear first-order compartment model. This model provides dynamic resistance such that increased resistance values signal constriction of the lungs. In these studies, 3 h following intranasal instillation of DE or saline, mice were anesthetized with a ketamine (166 mg/kg) and xylazine (8 mg/kg) cocktail, tracheostomized with a steel 18-gauge cannula, and mechanically ventilated at a rate of 160 breaths/min and tidal volume of 0.15 ml, using a computerized small animal ventilator (Finepoint; Buxco Electronics, Wilmington, NC) as previously described (27). Mice were allowed to stabilize on the ventilator for 5 min before measurements commenced. Once stabilized, dose responsiveness to aerosolized methacholine (1.5–48.0 mg/ml) was obtained and reported as total lung resistance.

Results

Dust-induced cellular inflammation. A single exposure to intranasal inhalation of swine confinement facility organic DE induced a significant increase in total leukocyte counts in lavage fluid compared with saline control 5 h after exposure (Fig. 1A,  , n = 8 mice/treatment group). There was a further increase in lavage fluid total cellularity after once-daily DE instillations for 1 and 2 wk compared with respective time-matched saline control (Fig. 1A). The increase in lavage cellularity was predominately due to expansion of neutrophils, but also macrophages (Fig. 1, B and C). These data demonstrate that an increase in inflammatory cells occurs immediately after single DE and that there is a progressive increase in inflammatory cells, predominately neutrophils, but to a lesser degree macrophages, over time with repetitive DE.

Dust-induced cytokine and chemokine response. To characterize the immune inflammatory response with single vs. repetitive intranasal DE, BAL fluid was assayed for the presence of neutrophil attractant CXC chemokines KC (CXCL1) and MIP-2 (CXCL2) and the cytokines TNFα and IL-6. There was a significant increase in TNFα, IL-6, KC, and MIP-2 5 h after single DE compared with saline control (Fig. 2, A–D; n = 8 mice/treatment group; , P < 0.05). Compared with saline control, TNFα, IL-6, KC, and MIP-2 levels in the lavage fluid remained significantly elevated after 2 wk (P < 0.05) of once-daily DE. However, there was a significant reduction of these cytokines/chemokines in the murine lavage fluid after 2 wk of DE compared with a single dust exposure (P < 0.05; n = 8 mice/treatment group). These findings demonstrate a consistent robust inflammatory response following single DE, and although diminished, a persistently elevated lavage inflammatory mediator response with repetitive DE.

Organic dust-induced PKCα and PKCe activity. Our previous work has demonstrated a significant role for PKC, specifically PKCα and PKCe isoforms in swine facility organic dust-induced inflammation in cultured bronchial epithelial cells (T. A. Wyatt, R. E. Slager, A. J. Heires, J. M. DeVasure, S. G. Von Essen, J. A. Poole, and D. J. Romberger, unpublished observations, and Ref. 32). In these current studies, we sought to determine whether DE in a murine model also modulated epithelial PKCα and PKCe activity. PKCα and PKCe activity was assayed in whole lung and isolated epithelial cells from trachea after single DE and 2 wk of once-daily DE, 5-h postintrasal exposure to DE, or saline as described in METHODS. As shown in Fig. 3, A and B, there was a robust activation of PKCα (13.3 ± 0.6-fold increase) and PKCe (19.9 ± 0.6-fold increase) in the isolated epithelial cells after single DE compared with control (P < 0.05; n = 6 mice/treatment group). Compared with control, after 2 wk of DE, PKCe activation remained significantly elevated (2.5 ± 0.5-fold increase; P < 0.01; Fig. 3D), but PKCα activity was not significantly elevated (1.6 ± 0.4-fold increase; P > 0.15; Fig.
However, activation of PKC\(\alpha\) and PKC\(\varepsilon\) was significantly dampened after 2 wk of DE compared with single DE (\(P < 0.05\)). In the whole lung tissues (a multicellular organ response), there was no change in PKC\(\alpha\) (1.0 \(\pm\) 0.08-fold increase) and PKC\(\varepsilon\) (1.0 \(\pm\) 0.04-fold increase) after single DE compared with saline control (\(n = 6\) mice/group). After 2 wk, there was a small but statistically significant increase in whole lung tissue PKC\(\alpha\) (1.4 \(\pm\) 0.1-fold increase, \(P = 0.03\), \(n = 6\) mice/group) in the DE mice compared with saline control, but no significant change in PKC\(\varepsilon\) (1.4 \(\pm\) 0.2-fold increase, \(P > 0.05\), \(n = 6\) mice/group).

These results in the isolated epithelial cells from the trachea are used to model lung epithelial cell responses and are consistent with prior in vitro findings in cultured bronchial epithelial cells (32). Moreover, these data paralleled cytokine/chemokine data in lavage fluid and suggest an important role for PKC\(\alpha\) and PKC\(\varepsilon\) in modulating dust-induced inflammation.

**Organic dust modulates alveolar macrophage function.** Previous in vitro studies have found that repetitive organic DE exposure significantly impairs the function of macrophages derived from monocytes (30). In these studies, aM\(\phi\) from the lavage fluid of the repetitive (2 wk) saline control and dust-exposed mice were collected, and functional studies as described in METHODS were conducted. Alveolar macrophages from the dust-treated mouse group stimulated with DE (1%) for 5 h demonstrated a modulated inflammatory response compared with the saline control group (Fig. 4A). Specifically, TNF\(\alpha\), KC, and MIP-2 levels were diminished in the 2-wk dust-exposed mouse group, but IL-6 levels were increased compared with the control group (pooled aM\(\phi\), \(n = 8\) mice/group). The phagocytic ability of the aM\(\phi\) from the dust-exposed mouse group was also reduced compared with the control group. These data suggest that repetitive dust exposure affects the function of the aM\(\phi\) in the lung lavage fluid, consistent with an impaired response.

**Dust-induced lung inflammation progresses with repetitive exposures.** To determine the effect on the lung parenchyma following dust exposure, formalin-fixed, paraffin-embedded whole lungs were sectioned and stained with H&E. The lung histology was notable for increased inflammatory cells within the bronchiolar and alveolar compartments with increasing duration of dust exposure compared with saline control [Fig. 5A depicts saline control and Fig. 5B depicts 12.5% DE histology changes after single and repetitive dust exposures (1 and 2 wk)]. Predominately perivascular and peribronchiolar mixed mononuclear cellular aggregates were consistently observed after 2 wk of daily intranasal dust exposure (Fig. 5C) and were not observed in saline-exposed mice. Microscopic review of the lung tissue demonstrated varying degrees of alveolar and bronchiolar inflammation as well as a spectrum of mononuclear cell aggregates in the dust-exposed mice. Although there were varying degrees of inflammation, no alveolar destructive process (emphysema) was identified in any of the animals.

To semiquantitatively assess the range of dust-induced histopathological changes, six mice per group were challenged with saline or DE (1%, 5%, 12.5%) once daily for 2 wk, and the pathology inflammatory scores were determined by a reviewer (pathologist) blinded to the treatment conditions as described in METHODS. There was a concentration-dependent effect in the semiquantitatively graded distribution of lung alveolar inflammation (Fig. 6A), bronchiolar inflammation (Fig. 6B), and mononuclear cellular aggregates (Fig. 6C). Lung inflammation was not observed with 1% DE but was significantly observed at the 5% and 12.5% DE concentration. These observations demonstrate significant, semiquantifiable lung pathology occurring with repetitive dust exposures.
Heterogeneity of dust-induced mononuclear cellular aggregates. To determine the composition of these organic dust-induced mononuclear cellular aggregates observed after 2 wk of daily DE exposure, formalin-fixed, paraffin-embedded slides were analyzed for CD3 (pan-T cell marker), CD45R/B220 (pan-B cell murine marker), and Mac-3 (phagocytes) by immunohistochemistry as outlined in METHODS. As shown in Fig. 7, mononuclear cellular aggregates were heterogenous, representing a mixture of T lymphocytes, B lymphocytes, and phagocytes. The majority of aggregates contained T lymphocytes and phagocytes with a smaller proportion of B lymphocytes (Fig. 7A). However, other mononuclear cellular aggregates observed were composed of primarily T and B lymphocytes with a smaller fraction of phagocytes (Fig. 7B). These data demonstrate that the novel mononuclear cellular aggregates observed with repetitive dust exposure are heterogeneous, predominately characterized by T lymphocytes and macrophages, and, to a lesser extent, B lymphocytes.

Dust-induced AHR. To determine if there were changes in lung function in response to single vs. repetitive intranasal DE vs. saline exposure, mice were assessed for AHR to methacholine by invasive pulmonary measurements, and there was a significant increase in AHR after single exposure to DE (12.5%) compared with saline-exposed controls (Fig. 8; 24 mg/ml and 48 mg/ml methacholine dose; P < 0.05; n = 4 mice/treatment group). However, after 2 wk, there was no change in AHR among the DE vs. saline (control)-treated mice. These results are consistent with an adapted response reported by others with swine barn air exposure (8).

DISCUSSION

Organic dust is an important agriculture environmental exposure that has been implicated in increased morbidity and lung disease among repetitively exposed subjects. Exposed individuals are at increased risk for developing significant respiratory diseases, particularly chronic bronchitis and obstructive pulmonary disease (37). In this study, a murine model utilizing single and repetitive intranasal inhalation of swine facility organic DE resulted in a reproducible, activated inflammatory airway response. A one-time exposure to dust resulted in significant increases in cellular influx, airway cytokine/chemokine release, and AHR. As hypothesized, airway mediator release dampened with repetitive exposures compared with single dust exposure, but the proinflammatory mediators in the lavage fluid remained significantly elevated after repet-
itive dust exposure compared with control. There was also expansion of airway neutrophils and macrophages with quantifiable increases in lung parenchymal inflammation marked by dense foci (mononuclear cellular aggregates) after 2 wk of repetitive dust exposure. Furthermore, PKC isoform activation paralleled lung inflammation suggesting PKC activation is an important signaling enzyme in organic dust-induced lung inflammation.

Proinflammatory mediators in the airway are suggested to contribute to the development of respiratory disease in swine farmers (2, 37). We found that TNFα, IL-6, KC (CXCL1), and MIP-2 (CXCL2) were robustly elevated in dust-exposed mice compared with control animals after single exposure, and albeit significantly dampened, remained persistently elevated after 2 wk of exposure conditions. These cytokines/chemokines are potent inflammatory mediators responsible for inducing pyrexia, neutrophil recruitment, activation of airway epithelial cells, and direct bronchial hyperreactivity (4). Our results with the intranasal inhalation method are consistent with other murine models of environmental inhalation injury such as endotoxin and cigarette smoke whereby proinflammatory cytokine release in the lavage fluid occurs within hours after exposure and dampens over time (3, 5, 35). In comparison, the swine barn air animal model has failed to demonstrate increases in the lavage fluid cytokines evaluated (TNFα, IL-1β, and IL-6; Ref. 9). This current model produced a consistent increase in lavage fluid inflammatory mediators, mediators that are well recognized to be increased in humans with organic dust-induced airway disease. We suggest here that this model provides an exaggerated, reproducible inflammatory response to dust exposure and that this model will be informative to future investigations of potential mechanisms driving organic dust-induced airway diseases.

In subjects with organic dust-induced respiratory disease, the airway lavage fluid is marked predominately by neutrophils, but also to a lesser degree lymphocytes and macrophages (2, 37). This current animal model demonstrated enhanced neutrophils, and, to a lesser degree, macrophages following dust exposure. In contrast, the swine barn air animal model found resolution of lavage cellularity (neutrophils) with repetitive exposures instead of a progressive enhancement of cellularity over time (8, 9). Our intranasal model is entirely consistent with nebulizer inhalation injury animal models. For example, repetitive daily inhalation of endotoxin by nebulizer to mice results in an expansion of lavage cellularity (neutrophils) with repetitive exposures instead of a progressive enhancement of cellularity over time, not dampening (5). Although these experimental manipulations...

![Fig. 3. Isolated tracheal epithelial cell PKC activity in saline and DE (12.5%)-treated mice. PKCα (A) and PKCε (B) activity demonstrated 5 h after single inhalation of DE (12.5%) and saline in mice. PKCα (C) and PKCε (D) activity after 2 wk of once-daily DE exposure, 5 h after last dose. Error bars are SE (n = 6 mice minimum/group). *P < 0.05 is statistically significant of DE vs. saline-treated mice.]
in rodents over relatively short time intervals are not indicative of a real world human exposure (years of exposure), artificial animal models are important investigative tools for future mechanistic studies, and limitations are acknowledged. In addition, organic dust samples from various geographical locations may also contribute to variability in inflammatory outcomes.

There are several potential explanations for the increase or persistence of lavage neutrophils. First, it has been previously recognized that various agricultural organic DE directly exhibit chemotactic activity in vitro (6, 38, 39), and unpublished data from our laboratory also demonstrate that this swine facility dust directly results in neutrophil chemotaxis (data not shown). The mechanisms of these findings are not fully understood, but porcine IL-8 (45) and a leukotriene B-like component (6) (both neutrophil chemoattractants) have been directly detected in low levels in organic DE. Second, swine facility organic DE results in increased ICAM expression on epithelial cells (23), which would increase neutrophil adherence and thus their predominance in the airway. Indeed, we have pilot data demonstrating that organic dust directly enhances neutrophil adherence to epithelial cells (data not shown). Finally, albeit dampened, MIP-2 and KC are still significantly elevated after 2 wk of daily dust exposure compared with control, and, therefore, their role in neutrophil chemoattraction persists. Collectively, we suggest that the in vivo organic dust-induced airway neutrophil response is not completely dependent on the classic neutrophil chemoattractants, but may be a direct result of the dust itself.

Another potential explanation for the adaptation-like and/or modulated inflammatory response observed here may be due to the role of aMφ. Monocytes can be recruited to sites of inflammation, and depending on which maturation and differentiation factors are present in the airway milieu, differentiate into dendritic cell (DC) phenotypes or macrophages (17, 36). We have previously found that the presence of organic dust alters monocytes’ ability to differentiate into macrophages in cell culture as evident by impaired cytokine responsiveness and phagocytic ability (30). In this study, due to a limited number of lavage aMφ, aMφ were pooled from repetitively exposed (2-wk) saline control and DE mouse groups and studied for cytokine responsiveness and phagocytosis. Ex vivo stimulation of aMφ with DE resulted in a modulated immune response
marked by reduced TNFα, MIP-2, and KC, but IL-6 secretion was increased. These findings are interpreted as consistent with the “tolerant” or adaptation response. Reduction in TNFα is considered the universal characteristic of tolerance, whereas others have found either suppression or augmentation of IL-6 (43). The aMϕ phagocytic ability was also reduced in the 2-wk dust-treated group compared with the control group. We speculate that a reduction in aMϕ function could impair clearing of
the dust and/or neutrophils from the airway, and, as a result, contribute to the persistent inflammatory response observed.

In mice challenged for 2 wk with dust, significant increases in lung parenchymal macrophages, lymphocytes, and neutrophils with distinct mononuclear cellular aggregates were found. These mononuclear cellular aggregates have not been described before in other dust-induced airway inflammatory models, and they consisted mainly of T lymphocytes and macrophages, and, to lesser extent, B lymphocytes. Similar mononuclear inflammatory cell aggregates in murine lung have also been noted in transgenic mice overexpressing proinflammatory cytokines (12, 18, 40). Specifically, in transgenic mice overexpressing IL-1β and IL-6, lymphocytic infiltrates without macrophages are observed, but in mice overexpressing TNFα, a heterogeneity of mixed mononuclear cellular aggregates is observed. It is possible that the mixed, heterogeneous cellular aggregate in our dust-induced model is secondary to the variety of the mediators released. It is also possible that increases in epithelial and endothelial cell adhesion molecules might be playing a role in formation of these cellular aggregates. It is recognized that swine facility DE induces epithelial cell adhesion of lymphocytes through ICAM expression (23). Others have found dust-induced expression of intercellular adhesion molecules in cultured monocytes (7), and enhanced endothelial cell markers (sICAM) in blood samples from swine dust-exposed humans have been shown (16).

![Fig. 7](image-url). Heterogeneous mononuclear cellular aggregates observed in mice after 2 wk of once-daily intranasal inhalation with DE (12.5%). A and B depict a different, but representative (2 of 4 mice), serial 4- to 5-μm-thick lung section of a focal mononuclear aggregate stained with anti-CD3 antibody for T lymphocytes, anti-murine CD45R/B220 antibody for B lymphocytes, and anti-Mac-3 antibody for phagocytes.

![Fig. 8](image-url). Methacholine (MCh)-induced airway hyperresponsiveness following single and 2-wk repetitive intranasal inhalation of DE (12.5%) and saline in mice. Total lung resistance (Rₐ) was directly measured using a mechanically ventilated mouse system. Data are expressed as means with standard error bars (n = 4 mice/group). *P < 0.05 is statistically significant.
A possible mechanism by which this organic dust exposure may modulate inflammation is through PKC activation. Exposure of cultured human airway epithelial cells to swine facility DE in vitro augments epithelial cell PKCα and PKCε activity resulting in epithelial cell IL-6 and IL-8/CXCL8 release (32). Studies also suggest that PKCα mediates TNFα, IL-6, and IL-8 release in cultured human epithelial cells and that PKCε is involved in IL-8 release, but not IL-6 and TNFα (T. A. Wyatt, R. E. Slager, A. J. Heires, J. M. DeVasure, S. G. Von Essen, J. A. Poole, and D. J. Romberger, unpublished observations).

In this in vivo study, there was a robust activation of PKCα and PKCε after single exposure to DE in isolated epithelial cells, but after 2 wk, this response was dampened. However, while dampened, PKCε remained significantly activated compared with saline control after 2 wk of dust exposure. Because of their accessibility, isolated epithelial cells from the trachea were used to model bronchial and alveolar epithelial cell responses throughout the lung. It is noted that studies with whole lung tissue were less informative as total PKC activity from the entire organ is likely composed of variable states of PKC activity in the numerous cell types of the lung. Still, PKCα was slightly increased in the 2-wk dust-treated murine whole lung tissue compared with saline control. Together, we propose that PKCα and PKCε play a significant role in organic dust-induced inflammatory responses in the airway and may represent targets for future therapeutic strategies.

When naive individuals are exposed to swine barns, there is a marked decline in postexposure peak flows and forced expiratory volume in 1-s (FEV1) with an associated increase in respiratory symptoms after a single exposure (19, 20). In contrast, swine farmers (repetitively exposed workers) do not experience such pronounced fluctuations in air flow or respiratory symptoms after exposure (28). Our murine model was consistent with the swine barn air model (8) in that single dust exposure evoked airway AHR to methacholine challenge and that this response was attenuated after 2 wk of repetitive dust exposure. The mechanism of organic dust-induced increases in AHR is not known. Neutrophil recruitment (33) and TNFα (29) can result in AHR, but AHR can also occur independently of neutrophil recruitment or TNF in other inflammatory models (21). In this study, increased airway responsiveness to swine barn dust inhalation corresponded to increases in airway lavage TNFα, IL-6, KC, and MIP-2 secretion, but not to cellular TNFα/H9251.

In conclusion, single and repetitive intranasal exposure to swine animal confinement facility DE in mice resulted in a reproducible model of exaggerated airway inflammatory and pathological injury. This model will allow qualitative and quantitative measures of dust-induced inflammation that may be useful for generating hypotheses of mechanistic regulation of environment-triggered respiratory disease. One potential target includes the PKC isoform(s) PKCα and PKCε. Furthermore, this murine model appears to mimic the well-recognized adaptation response described in humans.

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