Hog barn dust extract augments lymphocyte adhesion to human airway epithelial cells

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Mathisen, T., S. G. Von Essen, T. A. Wyatt, and D. J. Romberger. Hog barn dust extract augments lymphocyte adhesion to human airway epithelial cells. J Appl Physiol 96: 1738–1744, 2004. First published January 16, 2004; 10.1152/japplphysiol.00384.2003.—The dust of hog confinement facilities induces airway inflammation. Mechanisms by which this dust modulates inflammation are not completely defined, although it is clear that exposure to dust can modulate both epithelial cell and inflammatory cell function. In this work, we demonstrate that airway epithelial cell (BEAS-2B) treatment with hog barn dust extract (HDE) results in augmentation of peripheral blood lymphocyte adhesion to epithelial cell cultures in vitro. The augmentation of lymphocyte adhesion to epithelial cells is dependent on the concentration of HDE and time of HDE exposure, with twofold increases observed by 3 h and maintained at 24 h. Similar results are seen with primary human bronchial epithelial cells in culture. Lymphocyte adhesion to epithelial cells is inhibited in a concentration-dependent fashion by the treatment of epithelial cells with antibody to intercellular adhesion molecule-1 (ICAM-1). In addition, HDE exposure of epithelial cells results in an approximate twofold increase in ICAM-1 expression as determined by flow cytometry analysis. Pretreatment of epithelial cells with a protein kinase C-α (PKC-α) inhibitor, Gö-6976, also inhibited subsequent lymphocyte adhesion to HDE-exposed epithelial cells. These data suggest that airway epithelial cell HDE exposure enhances subsequent lymphocyte adhesion to epithelial cells that is mediated in part by HDE modulation of ICAM-1 expression and PKC-α.

Peripheral blood lymphocytes; interferon-gamma; swine confinement workers

PERSONS EXPOSED TO HOG CONFINEMENT facilities develop significant airway inflammation as demonstrated by marked increases of granulocytes in both nasal and bronchoalveolar lavage (BAL) fluid as well as increases in cytokines, including interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor (TNF)-α (15, 37). This airway inflammation gives rise to symptoms, including those of chronic bronchitis, in workers in confinement facilities (4). Lymphocytes, particularly CD8 lymphocytes, are thought to play an important role in the pathogenesis of chronic bronchitis (8, 14). Importantly, BAL fluid from hog farmers also shows an increase in lymphocytes (28). In addition, BAL from healthy subjects exposed to hog barns has recently been shown to contain increased amounts of IL-17, which is thought to link T-cell activation to neutrophilic inflammation (13). Thus mechanisms regulating lymphocyte interactions with the airway epithelium may be relevant to a better understanding of the inflammatory response and respiratory symptoms of hog confinement workers.

Our laboratory and others have observed that an extract of hog barn dust (HDE) is a potent stimulus of IL-8 and IL-6 release of airway epithelial cells in vitro (27, 29). Our laboratory has also demonstrated that HDE augments airway epithelial cell protein kinase C (PKC) activation and that the PKC-α isoform appears to mediate hog barn dust-stimulated IL-8 and IL-6 release of these cells (29). The effect of hog barn dust on epithelial cell receptors that may be involved with inflammatory cell adhesion within the airway such as intercellular adhesion molecule-1 (ICAM-1; CD54) has not been defined, but increased soluble ICAM-1 has been observed in BAL fluid of persons exposed to hog barn dust (17). Airway epithelial cells express ICAM-1 that is modulated by a variety of substances such as bacterial products, ozone, and inflammatory mediators, especially interferon (INF)-γ (6, 20, 26, 31). In this context, we hypothesized that hog barn dust extract mediates lymphocyte adhesion to epithelial cells via mechanisms involving ICAM-1 and PKC. We demonstrate that HDE augments peripheral blood lymphocyte adhesion to cultured human airway epithelial cells and that this dust-induced lymphocyte adhesion to epithelial cells is mediated, at least in part, by epithelial cell ICAM-1. We also observe that hog barn dust-augmented lymphocyte adhesion is significantly reduced by inhibition of epithelial cell PKC-α.

METHODS

Cell preparation. BEAS-2B cells (American Type Culture Collection, Manassas, VA), a SV40-transformed human bronchial epithelial cell line, were grown to confluency and examined. BEAS-2B cells were plated on type I collagen (Vitrogen 100, Cohesion, Palo Alto, CA)-coated 96-well tissue culture-treated plates (Corning, Corning, NY). Cells were grown in culture in LHC-9-RPMI 1640 (2:1) mix and incubated at 37°C in 5% CO2-95% air. LHC-9 medium contains LHC basal medium (Biofluids, Rockville, MD), 0.5 μM phosphoethanolamine-ethanolamine (Sigma-Aldrich, St. Louis, MO), 0.11 mM calcium (Fisher, Springfield, NJ), 50 μg/ml penicillin and streptomycin, 50 U/ml bovine insulin (Sigma-Aldrich), 0.2 μg/ml fungizone (Invitrogen, Carlsbad, CA), 2 μg/ml fungizone (invitrogen), trace elements (18), 5 μg/ml bovine insulin (Sigma-Aldrich), 5 ng/ml epidermal growth factor (Upstate, Lake Placid, NY), 10 μg/ml bovine transferrin (Sigma-Aldrich), 10 nM 3,5’-triiodothyronine (Biofluids), bovine pituitary extract (50 μg/protein/ml; Pel Freeze, Rogers, AR), 0.2 μM hydrocortisone (Biofluids), 0.5 μg/ml epinephrine (Sigma-Aldrich), and 0.1 μg/ml retinoic acid (Biofluids). Epithelial cells were rinsed with RPMI 1640 and then exposed to HDE, recombinant human INF-γ (R&D systems, Minneapolis, MN), lipopolysaccharide (LPS) from Escherichia coli (Sigma-Aldrich), or anti-human...
ICAM-1 (Upstate) for up to 24 h. Epithelial cells were exposed to concentrations of HDE ranging from 0.1% to 5% for varying amounts of times (0.25–24 h). The 5% HDE was the concentration of HDE utilized in most experiments based on our laboratory’s previous observations of the effect of HDE on other epithelial cells functions, including PKC activation and cytokine release (29). Our laboratory’s previous work has also shown that there is no associated toxicity with HDE concentrations used in these experiments (up to 20% HDE has been utilized with no evidence of epithelial cell cytotoxicity as measured by lactate dehydrogenase release).

Primary human bronchial epithelial cells (HBEC) were also utilized. These cells came from patients undergoing bronchoscopy for clinical reasons. After informed consent was obtained and with the approval of the Human Studies Subcommittee of the Research and Development Committee of the Omaha Veterans Affairs Medical Center, bronchoscopy brushings were obtained and processed using the technique of Kelsen et al. (11). These cells were grown in the same medium as the BEAS-2B cells. HDE was placed on the cells for time-dependent and dose-response assays. Similar results were seen with BEAS-2B and the primary bronchial epithelial cells.

**Lymphocyte preparation.** Peripheral blood lymphocytes were obtained by sedimentation method by using Accu-Prep lymphocytes (Accurate Chemical & Scientific, Westbury, NY). Lymphocytes were obtained from normal volunteers on a protocol approved by the Human Studies Subcommittee of the Research and Development Committee of the Omaha Veterans Affairs Medical Center. Coomassie blue stain revealed that the cells obtained via lymphocyte preparation were on average 95% lymphocytes. Lymphocytes had a >95% viability when stained with Trypan blue. Lymphocytes were labeled with calcein AM (Molecular Probes, Eugene, OR) for 1 h at room temperature. The cells were centrifuged and rinsed twice in RPMI and then activated with 50 ng/ml of phorbol dibutyrate (Sigma-Aldrich) for 15 min before use in experiment.

**Lymphocyte adherence assay.** Epithelial cells were rinsed three times with RPMI after exposure to various agents, lymphocytes were added at a concentration of 3 × 10^6/well, and cells were allowed to adhere for 20 min at 37°C. Nonadherent lymphocytes were removed by rinsing the wells one time with RPMI and two times with Dulbecco’s PBS (Invitrogen). Adherent cells were lysed with 1% Triton X-100 and read on a fluorometer (Fluorolite 1000, Thermo LabSystems, Beverly, MA) with an excitation wavelength of 490 nm and an emission wavelength of 530 nm. Quantitation of fluorescence was calculated by converting the number of adherent lymphocytes per well by using a standard curve of fluorescently labeled lymphocytes vs. fluorescence.

**Preparation of HDE.** Settled dust from hog confinement facilities was utilized for these experiments as previously described (29). The HDE was prepared by placing 1 g of dust in 10 ml of Hanks’ balanced salt solution without calcium (Biolofts). The mixture was vortexed and allowed to stand at room temperature for 1 h. The mixture was centrifuged for 10 min, and the supernatant was recovered and centrifuged again. The final supernatant was filter sterilized (0.22 μm) and used immediately.

The amount of endotoxin present in the HDE was measured by the Limulus amebocyte lysate kit (Biowhittaker, Walkersville, MD).

**Antibody treatment to epithelial cells.** BEAS-2B cells were grown to a confluent monolayer of cells in 96-well dishes coated with type I collagen. RPMI or 5% HDE was placed on the cells and incubated at 37°C for 24 h. Media were removed from cells and rinsed two times in RPMI before being incubated with or without anti-human ICAM-1 antibody (Upstate) for 1 h. Lymphocyte adherence assay was then performed.

**Flow cytometry analysis for epithelial cells.** BEAS-2B cells were plated on type I collagen-coated six-well plates (Falcon, BD Biosciences Discovery Labware, Bedford, MA) and grown to confluency. Cells were then exposed to control medium (RPMI) or 5% HDE for 24 h. With use of a standard trypsinization technique, the cell layer was harvested and trypsinization stopped by the addition of 0.1 ml of 0.2% soybean trypsin inhibitor (Sigma-Aldrich). Each sample was washed in 1 ml of sterile PBS and centrifuged two times. Cell pellets were resuspended in 1 ml of 0.2% dry milk in PBS and set for 30 min at room temperature on a rocker. Samples were then centrifuged and stained with either anti-human CD54-phycocerythrin (PE)-conjugated monoclonal antibody (BD PharMingen, San Diego, CA) or with a control R-PE-conjugated mouse IgG1 monoclonal immunoglobulin isotype control (BD PharMingen) at a concentration of 1:1,000 for 1 h at room temperature. Cells were centrifuged and rinsed with PBS, fixed with 1 ml of 1% paraformaldehyde, and centrifuged. Cell pellet was resuspended in 1 ml of PBS for analysis. Flow cytometry was performed by FACSCalibur (Becton Dickinson, Sunnyvale, CA). Mean fluorescence intensity was reported on 10,000 gated events for each sample.

**Inhibition of PKC-α.** BEAS-2B cells were grown to confluency on type I collagen-coated 96-well plates. The cells were rinsed with RPMI, and Gö-6976, a PKC-α inhibitor, was placed on the cells and incubated at 37°C for 1 h at a concentration of 1 μM. At this concentration, bronchial epithelial cells have been shown to have a cell viability of 95% or greater (9). After incubation, 10 μl of RPMI (control medium) or HDE were added to each well and incubated for 3 h. The lymphocyte adherence assay was then performed.

**Statistical analysis.** All experiments were performed no less than three separate times unless otherwise noted, and representative data of these experiments are presented. Data were analyzed for statistical significance by ANOVA utilizing Statview statistical program.

**RESULTS**

We observed that exposure of bronchial epithelial cells to HDE augments subsequent peripheral blood lymphocyte adhesion to the epithelial cells (Fig. 1). Confluent monolayers of epithelial cells were exposed to 5% HDE, IFN-γ (100 U/ml), or control media for 24 h. INF-γ was utilized for comparison because it is known that pretreatment of epithelial cells with

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**Fig. 1.** Peripheral blood lymphocyte adhesion to bronchial epithelial cell pretreated with hog barn dust extract (HDE) or interferon (IFN)-γ. BEAS-2B cells were treated with control media, 5% HDE, or 100 U/ml IFN-γ for 24 h. Fluorescently labeled lymphocytes (3 × 10^6/well) were allowed to adhere for 20 min at 37°C. Nonadherent lymphocytes were removed. Adherent cells were lysed with 1% Triton X-100 and read with a fluorometer. Vertical axis represents means ± SE of percent lymphocyte adhesion to epithelial cell monolayers (n = 4 experiments). *Significant P value by ANOVA.
pretreatment with IFN-γ/H9253 as opposed to the lymphocytes, due predominantly to the effect of HDE on the epithelial cells. HDE exposure demonstrates that pretreatment of the epithelial cells with HDE augments lymphocyte adhesion (26). Figure 1 demonstrates that pretreatment of the epithelial cells with HDE results in a twofold increase in subsequent lymphocyte adhesion compared with control, untreated cells (control cells, 4.1 ± 0.2%; HDE cells, 8.3 ± 1.2%; \( P = 0.003 \) by ANOVA). Pretreatment with IFN-γ also significantly increased adhesion (6.9 ± 1.2%, \( P = 0.03 \)).

We have also performed experiments using primary HBEC that are obtained at the time of bronchoscopy. Similar to the BEAS-2B cells, treatment of HBEC with 5% HDE for 24 h causes a twofold increase in peripheral blood lymphocyte adhesion. Lymphocyte adhesion to control, unstimulated HBEC was typically ~5% and increased to over 10% with HDE exposure.

To ascertain that the increase of lymphocyte adhesion was due predominantly to the effect of HDE on the epithelial cells as opposed to the lymphocytes, fluorescently labeled lymphocytes, which were activated with phorbol dibutyrate, were exposed to 5% HDE for 20 min (the amount of time the lymphocytes were in contact with the epithelial cells in assays in Fig. 1) and adhered to untreated epithelial cells. This did not induce an increase of lymphocyte adhesion to the epithelial cells (data not shown).

The increase in lymphocyte adhesion to HDE-treated bronchial epithelial cells is dependent on the length of time of the HDE exposure (Fig. 2). Bronchial epithelial cells were stimulated with 5% HDE for 0.25–24 h. There is no significant increase in adhesion over control untreated epithelial cells at time points of 0.25 h (4.9 ± 1.5%) and 2 h (8.2 ± 0.2%). However, after 3 h of HDE exposure, there is a significant increase in lymphocyte adhesion (11.05 ± 0.54%; \( P = 0.05 \)), which is sustained after 24 h of HDE exposure (14.7 ± 5.1%; \( P = 0.01 \)).

We also observed that HDE exposure of bronchial epithelial cells augments subsequent lymphocyte adhesion in a concentration-dependent fashion (Fig. 3). Bronchial epithelial cells were exposed for 24 h to concentrations of HDE ranging from 0.1 to 5%. A significant increase in adhesion is observed when the mean percent adherence of lymphocytes is compared with that of untreated epithelial cells (12.75 ± 1.2%) to 1% HDE (17.5 ± 1.1%; \( P = 0.01 \)) and 5% HDE (20.4 ± 1.6%; \( P < 0.0001 \)).

The lymphocyte mean percent adherence value for 0.1% HDE is 15.4 ± 0.98%, which is not significant compared with untreated epithelial cells. However, there is a significant increase (\( P = 0.007 \)) when 0.1% HDE is compared with 5% HDE.

Bacterial endotoxins are an important component in environmental organic dusts. In an attempt to identify whether endotoxin is predominantly responsible for the increase in lymphocyte adhesion to epithelial cells, we measured the amount of endotoxin in 5% HDE samples (by the Limulus amebocyte lysate kit) and then exposed the epithelial cells to LPS at the same concentrations. Repeatedly, LPS alone, at concentrations ranging from 97 to 194 ng/ml (the amount of endotoxin found in the HDE samples utilized) did not increase lymphocyte adhesion (see Fig. 4). The mean percent lymphocyte adhesion to untreated epithelial cells in this set of experiments is 8.98 ± 0.73%. Cells treated with LPS had a similar amount of adhesion (8.56 ± 0.72%). Cells treated with 5% HDE demonstrated the typical twofold increase in adhesion with 18.77 ± 1.45% (\( n = 3 \) experiments; \( P < 0.001 \) for 5% HDE compared with either control or LPS). Experiments utilizing higher concentrations of LPS (1–100 μg/ml) did not show a significant increase in lymphocyte adhesion to epithelial cell monolayers.

*Significant value by ANOVA.
augment lymphocyte adhesion minimally over control but was not statistically significant (data not shown). When HDE is applied to a polymyxin B column to remove endotoxin from the sample, no differences in lymphocyte adhesion to epithelial cells exposed to HDE or detoxified HDE (after the polymyxin B) were observed (data not shown), suggesting that endotoxin is not predominantly responsible for the HDE augmentation of lymphocyte adhesion to epithelial cells.

Airway epithelial cells express ICAM-1, which previously has been shown to mediate lymphocyte adhesion to the epithelium (26, 34). Therefore, we postulated that HDE augmentation of lymphocyte adhesion may be, at least in part, due to ICAM-1. HDE-exposed bronchial epithelial cells treated with antibody to ICAM-1 inhibits lymphocyte adhesion to HDE-exposed epithelial cells in a concentration-dependent manner (Fig. 5). The antibody concentrations utilized were 800 and 1,600 ng/ml (concentrations chosen were based on preliminary experiments using concentrations ranging from 400 to 1,600 ng/ml). Mean percent adherence to control untreated cells vs. 5% HDE-exposed cells is 8.05 ± 0.12 and 20.75 ± 2.93% (P = 0.0014; n = 2 experiments). Treating with 800 ng/ml antibody significantly reduces lymphocyte adhesion to HDE-exposed epithelial cells to 13.95 ± 2.26% (P = 0.024), and 1,600 ng/ml antibody further reduces lymphocyte adhesion to 9.00 ± 1.25% (P = 0.021). ICAM-1 antibody has no significant effect on lymphocyte adhesion to untreated epithelial cells (untreated control = 8.05 ± 0.12%; control + 800 ng/ml = 7.54 ± 0.50%; control + 1,600 ng/ml = 4.43 ± 0.25%)

We also observed that 5% HDE results in a significant increase in ICAM-1 expression of bronchial epithelial cells by flow cytometry analysis (Fig. 6). There is a clear shift in the histogram of epithelial cells exposed to 5% HDE for 24 h show a mean fluorescence intensity of 708.7 ± 73 (P = 0.004, Student’s t-test). This suggests that HDE enhances ICAM-1 expression on epithelial cells.

Our laboratory has previously observed that HDE stimulates airway epithelial cell PKC activation that results in IL-6 and 

![Fig. 4. Peripheral blood lymphocyte adhesion to bronchial epithelial cells exposed to lipopolysaccharide (LPS) in the same concentration as the amount of endotoxin present in the HDE. Epithelial cells were exposed to either 5% HDE or LPS at same concentration as measured in the HDE sample for 24 h before lymphocyte adhesion assay. Vertical axis represents means ± SE of percent lymphocyte adhesion to epithelial cell monolayers (n = 3 experiments). *Significant P value by ANOVA (P < 0.001).](image)

![Fig. 5. Anti-human intercellular adhesion molecule-1 (ICAM-1) antibody inhibits lymphocyte adhesion to HDE-exposed epithelial cells in a concentration-dependent manner. After exposure to either 5% HDE or control medium, epithelial cells were subject to 800 or 1,600 ng/ml of anti-human ICAM-1 antibody. Vertical axis represents means ± SE of percent lymphocyte adhesion to epithelial cell monolayers (n = 2 experiments). *Significant P value by ANOVA.](image)

![Fig. 6. ICAM-1 expression is significantly increased on bronchial epithelial cells after exposure to HDE. BEAS-2B cells were grown to confluency and exposed to control medium or 5% HDE for 24 h. Cell monolayers were harvested and stained with anti-human CD54-phycocyanin (PE)-conjugated monoclonal antibody. Flow cytometry was performed, and a shift in the histogram is present when the epithelial cells are exposed to HDE. Unstained cells (background) = 1, control cells = 2, and cells exposed to 5% HDE for 24 h.](image)
IL-8 release (29). Thus we postulated that PKC may also be involved with HDE augmentation of lymphocyte adhesion to epithelial cells. To examine this, we pretreated epithelial cells with a PKC-α inhibitor, Gö-6976, and subsequently exposed the epithelial cells to HDE for 3 h before performing the lymphocyte adhesion assay. The concentration of PKC-α inhibitor used was based on our previous data that showed that 1 μM effectively reduced HDE-augmented PKC activity and subsequent cytokine release. As shown in Fig. 7, 5% HDE resulted in the expected increase of lymphocyte adhesion (13.9 ± 1.2%; n = 2 experiments) compared with untreated cells (7.6 ± 0.8%; P < 0.0001). Pretreatment of epithelial cells with the PKC inhibitor and subsequently exposing the epithelial cells to HDE significantly reduced lymphocyte adhesion to nearly control values (6.7 ± 0.5%; P < 0.0001). PKC inhibition also resulted in a reduction in lymphocyte adhesion to control, unstimulated cells as well (2.4 ± 0.5%, P < 0.0001), suggesting the importance of constitutive PKC activation in mediating lymphocyte adhesion to epithelial cells. However, the effect of PKC-α inhibition on lymphocyte adhesion to unstimulated epithelial cells may not all be directly via ICAM-1 expression. When we assessed ICAM-1 expression by flow cytometry analysis, cells exposed to the PKC-α inhibitor (without HDE) did not demonstrate a significant reduction in ICAM-1 expression compared with untreated epithelial cells (data not shown). This implies that lymphocyte adhesion to unstimulated epithelial cells may utilize receptors in addition to ICAM-1. Our data do suggest that HDE augmentation of PKC activity is involved with HDE-induced lymphocyte adhesion to airway epithelial cells.

**DISCUSSION**

The dust of hog confinement facilities induces airway inflammation (7, 16). Mechanisms by which this dust modulates inflammation are not completely understood, although it is clear that exposure to confinement facilities can activate both epithelial cells and inflammatory cells (23, 27). In this study, we have demonstrated that epithelial cell-lymphocyte interactions are modulated by dust exposure. Specifically, we have observed that peripheral blood lymphocyte adhesion to bronchial epithelial cells in vitro is enhanced when epithelial cells are exposed to HDE in a dose- and time-dependent manner. We have shown that this augmentation of lymphocyte adhesion is, at least in part, mediated via ICAM-1 expression on epithelial cells and that HDE-induced PKC activation also modulates the lymphocyte adhesion.

Exposure to hog confinement facilities is clearly associated with evidence of airway inflammation in both naive subjects as well as workers (15, 17, 21). This airway inflammation likely contributes to the development of respiratory symptoms such as cough and a decline in lung function observed in workers (4, 30). There are increased numbers of lymphocytes and neutrophils in bronchoalveolar lavage (BAL) fluid of persons working in hog barns (28). Naive subjects also demonstrate an increase in lymphocytes and neutrophils in BAL fluid (16, 23), along with an increase in cytokines, including IL-8, IL-6, and TNF-α, after being in the environment for only a few hours (15, 37). Our experiments suggest the possibility that lymphocytes within the airway may also have enhanced adhesion to dust-exposed epithelium and thus further modulate airway inflammatory responses. It remains to be determined whether dust modulation of lymphocyte interactions within the airway epithelium may be related to the concept of tolerance that is believed to occur in some workers in this environment (36).

Hog barn dust has been shown to activate airway epithelial cells in vitro, resulting in augmentation of IL-6 and IL-8 expression as well as PKC activation (27, 29, 38). The release of IL-6, IL-8, and other cytokines from epithelial cells may create cytokine gradients that influence inflammatory cell recruitment within the airway. Furthermore, Laan et al. (13) recently observed an increase in IL-17 in BAL of persons exposed to a hog confinement facility. IL-17 has been suggested to link activation of T lymphocytes to neutrophilic inflammation (19). Although IL-17 is predominantly produced by lymphocytes and eosinophils, it is capable of augmenting epithelial cell IL-6, IL-8, and ICAM-1 (10). Taken together, these data suggest that a variety of cytokines are present in the airway of persons exposed to hog confinement facilities. These cytokines likely interact to influence airway epithelial cell behavior, such as the augmentation of ICAM-1 expression induced by HDE and the subsequent enhancement of lymphocyte adhesion to epithelial cells demonstrated in the present.

![Fig. 7. Pretreatment of bronchial epithelial cells with Gö-6976 before stimulation of the cells with HDE significantly inhibits lymphocyte adhesion. BEAS-2B cells were treated with or without 1 μM Gö-6976 for 1 h and then exposed to control medium or 5% HDE for 3 h. Vertical axis represents means ± SE of percent lymphocyte adhesion to epithelial cell monolayers. *Significant P value by ANOVA.](http://jap.physiology.org/)

![Fig. 8. Schematic of the influence of HDE on airway epithelial cell function. PKC-α, protein kinase C-α; IL, interleukin.](http://jap.physiology.org/)
study. However, we have observed that neither IL-6 nor IL-8 in concentrations typically released by cultured airway epithelial cells exposed to HDE directly augment epithelial cell ICAM-1 expression (data not shown), implying that these two cytokines are not responsible for the changes in lymphocyte adhesion we observed.

Under our experimental conditions, we observed that HDE is augmenting lymphocyte adhesion to epithelial cells predominately via HDE effect on epithelial cells and not the lymphocytes. Specifically, we observed that when lymphocytes are stimulated with HDE for 20 min and epithelial cells are not exposed to HDE, there is no increase in lymphocyte adhesion (data not shown). Clearly, organic dusts such as hog barn dust may modulate lymphocyte function. Müller-Suur and colleagues (24, 25) have shown lymphocyte activation ex vivo as determined by increased expression of markers CD69, CD25, and human leukocyte antigen (HLA)-DR when whole blood of healthy subjects is exposed to dust. They also observed that T lymphocytes were only activated in the presence of phagocytic cells (25). We observed an increase of lymphocyte adhesion when lymphocytes were exposed to HDE for a longer time period (24 h) before the adhesion assay was performed (data not shown). Thus hog barn dust may have multiple effects on lymphocyte activation, especially in vivo. However, within the first 24 h of these adhesion assays, the HDE appeared to be primarily influencing the epithelial cells.

Endotoxin is a known component of dusts found in hog barns (2, 32). Data from Vogelzang et al. (35) demonstrate that workers in hog farms have decreased lung function after exposure to endotoxin. However, others have observed that neither endotoxin alone nor total dust nor ammonia appears to be solely responsible for pulmonary inflammation or symptoms in persons exposed to hog barns (5, 16). Interestingly, our laboratory and others observed that endotoxin is not as potent as hog barn dust in stimulating cytokine release from airway epithelial cells (27, 29, 38). In the present study, we observed that LPS, in the same concentration as the amount of endotoxin measured in the HDE, does not augment lymphocyte adhesion. This suggests that components of HDE other than endotoxin are likely responsible for mediating lymphocyte adhesion to epithelial cells. We have not yet identified the component(s) of HDE that is most potent in modulating the adhesion.

ICAM-1, a cell-surface glycoprotein on bronchial epithelial cells, binds to lymphocyte function-associated antigen-1 (LFA-1) on inflammatory cells, including lymphocytes. The importance of ICAM-1 in adhesion and transmigration of T lymphocytes in the airway epithelium has been demonstrated both in vivo in asthmatic tissue and in vitro (26, 33). Similarly, the importance of ICAM-1 in mediating lymphocyte adhesion has also been seen with epithelial cells from other organs (34). We observed that HDE directly augments ICAM-1 expression of airway epithelial cells as measured by flow cytometry. Furthermore, we observed that HDE-augmented lymphocyte adhesion to epithelial cells is reduced to nearly the level of untreated epithelial cells with increasing concentrations of blocking antibody to ICAM-1 (Fig. 6). These data support an important role of HDE-augmented ICAM-1 expression in regulating lymphocyte adhesion to epithelial cells. We have not directly excluded that HDE may not have an effect on additional epithelial receptors that can influence lymphocyte adhesion such as CD44, LFA-3, or β6-integrin subunit that we have not examined in these studies (1, 22).

Our laboratory has previously observed that HDE augments PKC activation of airway epithelial cells that modulates IL-8 and IL-6 release (29). PKC activation has also been shown to influence epithelial cell ICAM-1 expression as well. Krunksky et al. (12) have shown that TNF-α mediates ICAM-1 expression of bronchial epithelial cells via a PKC-dependent mechanism. Additionally, Chang et al. (3) demonstrated that PKC-dependent c-Src tyrosine kinase activation pathway modulates INF-γ-induced ICAM-1 expression of airway epithelial cells and subsequent monocyte adhesion. We also observed that PKC modulates HDE-augmented lymphocyte adhesion (Fig. 7). We used a PKC-α isoform inhibitor because others have shown the importance of this isoform in mediating cytokine release of airway epithelial cells (9, 39). However, others have suggested that the calcium-independent, novel, or atypical PKC isoforms rather than the classic calcium-dependent isoforms such as PKC-α may be important in TNF-α modulation of ICAM-1 expression of airway epithelial cells (12).

In summary, we have observed that an extract of dust from hog confinement facilities augments peripheral blood lymphocyte adhesion to airway epithelial cells in vitro. Figure 8 summarizes our present concept of the effect of HDE on airway epithelial cell function. From our previous work, we know that HDE augments epithelial cell PKC activation, including the PKC-α isoform, which augments epithelial cell IL-8 and IL-6 release and influences inflammatory cell recruitment to the epithelium (29). In this study, we have demonstrated that HDE via PKC-α also augments epithelial cell ICAM-1 expression that augments inflammatory cell adhesion to the epithelium. Further understanding of mechanisms involved in airway inflammation in environments rich in organic dusts like hog confinement facilities may help define appropriate measures of in vivo monitoring of workers to better define those most at risk and to assess whether workplace prevention strategies are effectively reducing inflammation.

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