
Grain dust induces IL-8 production from bronchial epithelial cells: the effect of dexamethasone on IL-8 production

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Background: Recent publications have suggested an active participation of neutrophils to induce bronchoconstriction after inhalation of grain dust (GD).

Objective: To further understand the role of neutrophils in the pathogenesis of GD-induced asthma, this investigation was designed to determine whether human bronchial epithelial cells could produce IL-8 production and to observe the effect of dexamethasone on IL-8 production.

Materials and methods: We cultured Beas-2B, a bronchial epithelial cell line. To observe GD-induced responses, four concentrations (1 to 200 $\mu\text{g/mL}$) of GD were incubated for 24 hours and compared with those without incubation of GD. To evaluate the effect of pro-inflammatory cytokines on IL-8 production, epithelial cells were incubated with peripheral blood mononuclear cell (PBMC) culture supernatant, which was derived from the culture of PBMC from a GD-induced asthmatic subject under the exposure to 10 $\mu\text{g/mL}$ of GD, and compared with those cultured without addition of PBMC supernatant. The level of released IL-8 in the supernatant was measured by enzyme-linked immunosorbent assay. To evaluate the effect of dexamethasone on IL-8 production, four concentrations (5 to 5000 ng/mL) of dexamethasone were pre-incubated for 24 hours and the same experiments were repeated.

Results: There was significant production of IL-8 from bronchial epithelial cells with additions of GD in a dose-dependent manner ($P < .05$), which was significantly augmented with additions of PBMC supernatant ($P < .05$) at each concentration. Compared with the untreated sample, pretreatment of dexamethasone could induced a remarkable inhibitions (15% to 55%) of IL-8 production from bronchial epithelial cells in a dose-dependent manner.

Conclusion: These results suggest that IL-8 production from bronchial epithelial cells may contribute to neutrophil recruitment occurring in GD-induced airway inflammation. The downregulation of IL-8 production by dexamethasone from bronchial epithelial cells may contribute to the efficacy of this compound in reducing cellular infiltration and ultimately to its anti-inflammatory property.

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INTRODUCTION

Several mechanisms including IgE-mediated response may be involved in the pathogenesis of GD-induced occupational asthma.¹ Recent publications

have suggested a possible role of neutrophils in the development of GD-induced airway obstruction.²⁻⁴ The immunohistochemical study demonstrated that the neutrophil counts were significantly higher in the bronchial mucosa of GD-induced asthma than in that of allergic asthma.² The other study dealing with neutrophil chemotactic activity from subjects with GD-asthma³ revealed that serum neutrophil chemotactic activity significantly increased at 30 minutes after GD inhala-

tion challenge and then significantly decreased at 240 minutes, whereas no significant changes occurred in asthmatic patients with negative results on GD-bronchoprovocation test.

In order to evaluate the role of IL-8 on neutrophil recruitment in GD-induced asthma, sputum IL-8 levels were measured. Interleukin-8 level significantly increased after the GD-bronchial challenge test compared with the baseline level, whereas no significant changes occurred in asthmatic subjects who showed a negative result on GD-bronchoprovocation test.² These findings suggest that activated neutrophils may participate in the development of GD-induced bronchoconstriction in exposed workers, and IL-8 release after exposure to GD might contribute to the neutrophil infiltration into the airway mucosa.

Interleukin-8 has been recognized as an inflammatory cytokine with strong potency in neutrophil activation and chemotaxis.^{5,6} To the best of our knowledge, there has been little data concerning GD-induced cytokine production from bronchial epithelial cells. We therefore observed the production of IL-8 induced by GD from epithelial cells, and also investigated the effect of dexamethasone on IL-8.

MATERIALS AND METHOD

Preparation of Extracts

Grain dust was obtained from the subjects' workplace. It was extracted with phosphate-buffered saline [(PBS, pH 7.5), 1:5 wt/vol] at 4°C for 1 hour followed by centrifugation at 5,000 rpm. The supernatants were passed through the syringe filter (MIS, USA: 0.25 μm pore-sized), dialyzed (the cut-

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off molecular weight was 6,000 Da) against 4 L of distilled water at 4°C for 48 hours, and lyophilized at -70°C for the preparation of antigens which were used for stimulation of epithelial cells.

Cell Cultures

The Beas-2B cell line, derived from human bronchial epithelium transformed by an adenovirus 12-SV40 hybrid virus was purchased from ATCC (USA). Cells were cultured in 75-cm² tissue culture flasks and maintained in DMEM/F12 medium (Gibco, Gaithersburg, MD) containing 10% heat-inactivated FBS, penicillin (100 U/mL), and streptomycin (100 mg/mL, Sigma, St. Louis, MO). Cells were detached from the flasks with 0.05% trypsin, 0.53 mM EDTA 4 Na, 0.02% ethylene glycol-bis-tetra acetic acid (Sigma, St. Louis, MO) in DMEM/F12, and cultures were maintained until passing equivalent numbers of cells into 75-cm² flask, at which time 90% of surface were confluent. Cells were cultured at 37°C with 5% CO₂ in humidified air. Viability of cells, assessed by staining with trypan blue, was always ≥95% of cells harvested.

Experimental Design

To observe GD-induced response, bronchial epithelial cells (1 × 10⁶ per each well) were incubated with four concentrations of GD and compared with those without GD. For concentration-dependent experiments, cells were cultured with serial additions of GD (1, 10, 100, and 200 µg/mL) for 24 hours and the supernatants were collected.

Furthermore, to evaluate the effect of pro-inflammatory cytokine on IL-8 production, epithelial cells (1 × 10⁶ per each well) were cultured with PBMC supernatant according to the previously described method with some modifications.⁷ Peripheral blood mononuclear cells were purified using Ficoll-Paque (Pharmacia, Sweden) from one subject with GD-induced asthma. They (1 × 10⁶ cells) were incubated for 24 hours with 10 µg/mL of GD, which is an optimal concentration to stimulate T cell in the previous experiment.⁸ The supernatants were collected and were included in the epithelial cell culture medium (the ratio of the supernatant to DMEM/F12 was 1 to 3 vol/vol). For the inhibition studies by dexamethasone, bronchial epithelial cells were pre-exposed to four concentrations (5 to 5000 ng/mL) of dexamethasone (Daewon Pharmaceutical Co, Korea) for 24 hours under conditions of PBMC-containing culture medium (DMEM/F12), and then treated with 100 µg/mL of GD. After 24 hours, the supernatants were collected, centrifuged, and stored at -20°C for subsequent analysis by a specific ELISA for IL-8 protein. As control, an equivalent amount of diluent was added instead of dexamethasone. All the experiments were performed in six sets under the same experimental conditions. The peripheral blood mononuclear cell donor gave his informed consent regulated by Ajou University Hospital, Suwon, Korea.

ELISA for IL-8 Level

Levels of immunoreactive IL-8 were quantified using a homemade sandwich-type ELISA. ELISA plates (Costar, Cambridge, MI) were coated with a specific murine monoclonal against human IL-8 antibody (Endogen, Woburn, MI). Fifty microliters of a dilution (1:25 vol/vol) of cell-free supernatants, which was decided as optimal in the preliminary experiment, was added in triplicate, followed by the addition of a second streptavidin-peroxidase-conjugated goat polyclonal antibody against IL-8 antibody (Endogen, Woburn, MI). After washing to remove any unbound antibody-enzyme reagent, a substrate solution (0.05 M phosphate citrate buffer 10 mL, tetramethylbenzidine 1 mg, and 2 µL of hydrogen peroxide) was added to the wells. The color development was stopped with 2.5 N sulfuric acid. The intensity of the color was measured at 450 nm on a spectrophotometer. A standard curve was generated by plotting the optical density against the concentration of IL-8 in the standard wells, using a curve-fitting program. This ELISA had less than 10% of intra-assay coefficient variation.

Statistical Analysis

The Mann-Whitney U and Wilcoxon-signed rank tests were applied using SPSS version 7.0 (Chicago, IL) to evaluate the statistical differences between the data. A *P* value of less than .05 was regarded as significant. Data was expressed as mean ± SD.

RESULTS

Production of IL-8 from Bronchial Epithelial Cells and Effect of Peripheral Blood Mononuclear Cell Supernatant on IL-8 Production

As shown in Table 1, there was a significant increase of IL-8 production with additions of four different concentrations of GD (1 to 200 µg/mL), compared with controls which was derived from those with no addition of GD. Statistically significant increase of IL-8 production was noted with additions of GD in a dose-dependent manner (*P* < .05, respectively), in

Table 1. Interleukin-8 Production from Bronchial Epithelial Cells, Beas-2B with Additions of Grain Dust

Grain Dust IL-8, µg/mL Concentration, µg/mL	Peripheral Mononuclear Cell Supernatant	
	Without	With
0	1.32 ± 0.01*	2.66 ± 0.32*
1	1.80 ± 0.02	112.36 ± 8.28*
10	2.22 ± 0.12*	139.78 ± 8.86*
100	8.42 ± 0.58*	225.92 ± 8.16*
200	12.12 ± 0.19*	273.24 ± 25.3*

* *P* < .05, compared between control (IL-8 level without addition of grain dust) and those with serial addition of grain dust.

All the values were presented as mean and SD.

which they were significantly augmented with the addition of PBMC supernatant at each concentration of GD ($P < .05$, respectively). Interleukin-8 production was minimal without addition of GD.

Effect of Dexamethasone on IL-8 Production

To evaluate the influence of dexamethasone on IL-8 production, four concentrations (5 to 5000 $\mu\text{g/mL}$) of dexamethasone were pre-incubated for 24 hours before the addition of GD. Dexamethasone could remarkably inhibit IL-8 production in a dose-dependent manner, and the inhibition percent ranged from 15% to 55% as shown in Figure 1.

DISCUSSION

Recent investigations have suggested that neutrophils might be involved in the pathogenesis of GD-induced asthma.²⁻⁴ Inhalation of grain sorghum dust in normal volunteers could induce an increased peripheral neutrophil count and chemotactic response to GD.⁴ In vitro study revealed the release of substances presenting neutrophil chemotactic activity by bronchial epithelial cell in response to GD.⁹ So far, the mechanism of neutrophil chemotaxis and the function of the neutrophils in GD-induced asthma are not well documented. Interleukin-8 was recognized as a key chemokine to induce neutrophil recruitment and activation.^{5,6} Interleukin-8 is produced from various cells, but epithelial cell is one of the major sources of IL-8.¹⁰ The previous study dealing with IL-8 release in tracheobronchial secretion from the subjects with GD-induced asthma demonstrated that IL-8 level in the induced sputum significantly increased after the GD bronchoprovocation test, while no significant changes were noted in asthmatic subjects showing a negative result on GD-bronchoprovocation test.² These results suggest that IL-8 may contribute to neutrophil recruitment into airway when they are exposed to GD.

Epithelial cells lining the airways are in contact with a variety of agents

able to elicit a local inflammatory response, including viruses, allergens, and microbes. Recent studies now indicate that airway epithelium is not only a structural barrier, but it is also a source of pro-inflammatory mediators and cytokines able to modulate such inflammation.¹¹⁻¹⁵ Epithelium is damaged by the inflammatory process that occurs in allergic airway disease especially in asthma, resulting in functional impairment.^{16,17} Our previous study demonstrated that neutrophil infiltrations were noted in bronchial mucosa of subjects with GD-induced asthma³ and the IL-8 level in their induced sputum significantly increased after the GD-bronchoprovocation test. Based upon these findings, we could hypothesize that epithelial cells might be a source of IL-8 for recruiting neutrophils into airway mucosa. This study demonstrates that IL-8 production is minimal in unstimulated epithelial cell cultures; however, it was inducible with additions of GD antigen in a concentration-dependent manner.

There have been several reports showing that cytokine production from bronchial epithelial cells could be augmented by pro-inflammatory cytokine such as TNF- α and γ -IFN.^{10,18} Our previous study⁸ showed that purified T cells from subjects with GD-induced asthma produced γ -IFN when they are stimulated with the addition of GD antigens. In this study, IL-8 production was significantly augmented by PBMC

supernatant at each concentration of GD. This result may have been caused by pro-inflammatory cytokines, included in PBMC supernatant, which were released from PBMC when exposed to GD. These findings suggest that GD exposure may stimulate IL-8 release from bronchial epithelial cell in a direct manner, but also it may induce release of pro-inflammatory cytokine resulting in of IL-8 production from bronchial epithelial cells.

One of the important anti-inflammatory mechanisms by corticosteroids in allergic inflammation is derived from the inhibition of eosinophil recruitment, which inhibits the formation of eosinophil and endothelial-activating cytokines, the former including IL-5 and GM-CSF, and the latter including IL-1, TNF- α , and IL-4.¹⁹ Evidence is accumulating that glucocorticoid also inhibits the expression of epithelial-derived cytokines including IL-6, GM-CSF,²⁰ and IL-8.²¹ In this study, GD-induced IL-8 production from bronchial epithelial cells was sensitive to the action of glucocorticoids; dexamethasone could dose-dependently suppress GD-induced IL-8 production from epithelial cells. The molecular mechanism of dexamethasone-mediated inhibition on IL-8 production induced by GD has to be further clarified. Interleukin-8 is regulated by NF κ B, and glucocorticoid could increase expression of the gene for I κ B α

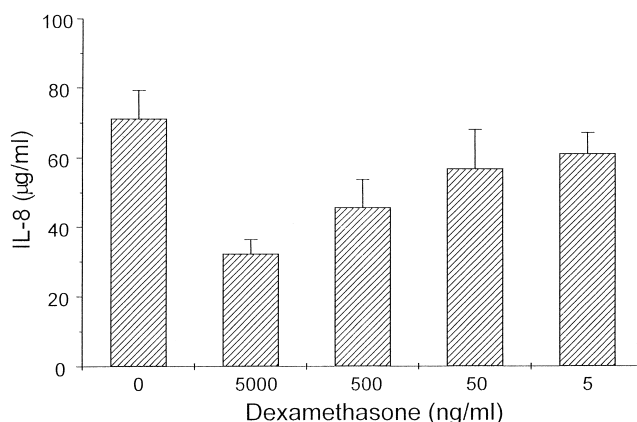


Figure 1. Effect of dexamethasone pretreatment on IL-8 production from bronchial epithelial cells, Beas-2B.

and protein, which binds activated NF- κ B.²²

In conclusion, IL-8 induced by GD from bronchial epithelial cells may contribute to neutrophil recruitment into airway mucosa when they are exposed to GD. Dexamethasone has a downregulatory effect on this cytokine production.

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REFERENCES

1. Park HS, Nahm DH, Suh CH, et al. Occupational asthma and IgE sensitization to grain dust in exposed workers. *J Kor Med Sci* 1998;13:275–280.
2. Park HS, Jung KS, Hwang SC, et al. Neutrophil infiltration and release of IL-8 in airway mucosa from subjects with grain dust-induced occupational asthma. *Clin Exp Allergy* 1998;28:724–730.
3. Park HS, Jung KS. Enhanced neutrophil chemotactic activity after bronchial challenge in subjects with grain dust-induced asthma. *Ann Allergy Asthma Immunol* 1998;80:257–262.
4. Von Essen SG, Robbins RA, Thompson AB, et al. Mechanisms of neutrophil recruitment to the lung by grain dust exposure. *Am Rev Respir Dis* 1998;138:921–927.
5. Suzuki H, Takahashe Y, Wataya H, et al. Mechanism of neutrophil recruitment induced by IL-8 in chronic sinusitis. *J Allergy Clin Immunol* 1996;98:659–670.
6. You A, Kitagawa S, Kasahara T, et al. Stimulation and priming of human neutrophils by interleukin-8: cooperation with tumor necrosis factor and colony-stimulating factors. *Blood* 1991;78:2708–2714.
7. Standiford TJ, Kunkel SL, Basha MA, et al. IL-8 gene expression by a pulmonary epithelial cell line. A model for cytokine networks in the lung. *J Clin Invest* 1990;86:1945–1953.
8. Lee M, Son M, Park S, et al. Grain dust reactive T cell produced IFN- γ grain dust induced occupational asthma subjects. *J Allergy Clin Immunol* 1997;99:S81.
9. VonEssen SG, O'Neill DP, Robbins RA, et al. Neutrophilic chemotaxis to extracts to grain plant component. *Am J Int Med* 1994;25:85–88.
10. Kwon OJ, AU BT, Collins PD, et al. Tumor necrosis factor-induced interleukin-8 expression in cultured human airway epithelial cells. *Am J Physiol* 1994;267:L398–405.
11. Churchill L, Chilton FH, Resau JH, et al. Cyclo-oxygenase metabolism of endogenous arachidonic acid by cultured human tracheal epithelial cells. *Am Rev Respir Dis* 1989;140:449–459.
12. Maini M, Vittori E, Hollemborg J, et al. Expression of the potent inflammatory cytokines, granulocyte-macrophage colony-stimulating factor and interleukin-6 and interleukin-8, in bronchial epithelial cells of patients with asthma. *J Allergy Clin Immunol* 1992;89:1001–1009.
13. Churchill L, Friedman B, Schleimer RP, et al. Production of granulocyte macrophage colony-stimulating factor by cultured human tracheal epithelial cells. *Immunology* 1992;75:189–195.
14. Kwon OJ, Au BT, Collins PD, et al. Inhibition of interleukin-8 expression by dexamethasone in human culture airway epithelial cells. *Immunol* 1994;81:389–394.
15. Sousa AR, Lane SJ, Nakhosteen JA, et al. Increased expression of the monocyte chemoattractant protein-1 in bronchial tissue from asthmatic subjects. *Am J Respir Cell Mol Biol* 1994;10:142–147.
16. O'Byrne PM, Adelroth E. Airway epithelial inflammation and its functional consequences. In: Goldie R, eds: *Immunopharmacology of epithelial barriers*. Avon, UK: The Bath Press, 1994; 150–157.
17. Montefort S, Herbert SCA, Robinson C, et al. The bronchial epithelium as a target for inflammatory attack in asthma. *Clin Exp Allergy* 1992;22:511–516.
18. Terada N, Maesako K, Hamano N, et al. RANTES production in nasal epithelial cells and endothelial cells. *J Allergy Clin Immunol* 1996;98:S230–237.
19. Scheleimer RP. Effects of glucocorticoids on inflammatory cells relevant to their therapeutic applications in asthma. *Am Rev Respir Dis* 1990;141:S59–69.
20. Levine SJ, Larivee P, Logun C, et al. Corticosteroids differentially regulate secretion of IL-6, IL-8, and G-CSF by a human bronchial epithelial cell line. *Am J Physiol* 1993;265:L360–368.
21. Standiford TJ, Kunkel SL, Gromer KR, et al. Regulation of interleukin 8 gene expression from human tracheal explant derived epithelial cells by dexamethasone. *Am Rev Respir Dis* 1992;145:A638.
22. Franklin HE, Peter JB, Michael K. Nuclear factor B-A pivotal transcription factor in chronic inflammatory disease. *N Engl J* 1997;10:1066–1071.

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