

## Exposure to Toluene Diisocyanate (TDI) Induces IL-8 Production from Bronchial Epithelial Cells: Effect of Pro-inflammatory Cytokines

This investigation was designed to confirm IL-8 production from human bronchial epithelial cells with toluene diisocyanate (TDI) exposure and to examine the effects of pro-inflammatory cytokine and dexamethasone. We cultured Beas-2B, a bronchial epithelial cell line with TDI-HSA conjugate and compared with those without conjugate. IL-8 in the supernatant was measured by ELISA. To evaluate the effect of pro-inflammatory cytokines, peripheral blood mononuclear cells (PBMC) were collected from TDI- and non-TDI asthma patients, and were added to the epithelial cell culture. Dexamethasone or antibodies to TNF- $\alpha$  and IL-1 $\beta$  were pre-incubated with PBMC supernatant. There was a significant production of IL-8 from bronchial epithelial cells with addition of TDI-HSA conjugate in a dose-dependent manner, which was significantly augmented with addition of PBMC supernatant. Higher production of IL-8 was noted with addition of PBMC supernatant from TDI-asthma patients than in those from non-TDI asthma patients. IL-1 $\beta$  and IL-1 $\beta$ /TFN $\alpha$  antibodies were able to suppress the IL-8 productions. Pre-treatment of dexamethasone induced dose-dependent inhibition of the IL-8 production. These results suggest that the IL-8 production from bronchial epithelial cells contribute to neutrophil recruitment occurring in TDI-induced airway inflammation. IL-1 $\beta$  released from PBMC of TDI-induced asthma patients may be one of the pro-inflammatory cytokines to enhance IL-8 production.

**Key Words :** Isocyanates; TDI-Asthma; Epithelial Cells; Interleukin-8; Interleukin-1; Dexamethasone

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## INTRODUCTION

Toluene diisocyanate (TDI) is a common cause of occupational asthma worldwide and is the leading cause of occupational asthma in Korea (1). Although there has been evidence suggesting involvement of cell-mediated immunity composed of activated lymphocytes, eosinophils and mast cells (2), a recent investigation employing immunohistochemical and induced sputum technology has demonstrated the possible involvement of activated neutrophil in TDI-induced bronchoconstriction and IL-8 production was increased after TDI exposure in the airway selection of TDI-induced asthma patients (3, 4).

IL-8 has been recognized as a strong chemokine for inducing neutrophil activation and chemotaxis (5). Epithelial cell was a major source of chemokines, especially IL-8 in response to exogenous stimuli in asthmatic subjects (6). The chemokine productions were augmented by pro-inflammatory cytokines including IL-1 $\beta$ , TNF- $\alpha$  and IFN- $\gamma$  in vitro culture of epithelial cells (7). To the best of our knowledge, there has been little data available on TDI-induced IL-8 production from bronchial epithelial cells and the effect of pro-inflammatory cytokines on this chemokine production. Therefore, we look

at IL-8 production; a representing chemokine for neutrophil, from the bronchial epithelial cells with and without TDI-HSA conjugate incubation and also investigated the effect of pro-inflammatory cytokines derived from PBMC culture supernatant of two different donor groups. The effect of dexamethasone as a representing anti-inflammatory agent for asthma treatment was evaluated on this IL-8 production.

## MATERIALS AND METHODS

### Experimental design

TDI-human serum albumin (HSA) conjugates were prepared as described in the previous report (8). The Beas-2B cell line, derived from human bronchial epithelium transformed by an adenovirus 12-SV40 hybrid virus was purchased from ATCC. Cells were cultured in BEGM medium (Clonetics, Walkersville, MD, U.S.A.) containing additives such as bovine pituitary extract, insulin, hydrocortisone, gentamicin at 37°C with 5% CO<sub>2</sub> in humidified air. To observe TDI-induced response, epithelial cells were incubated with four concentrations (0.5 to 500  $\mu$ g/mL) of TDI-HSA conjugate for 24

hr and compared with those without incubation, and then the supernatants were collected. Two groups of PBMC donors were enrolled; for TDI asthma donor, bronchial sensitizations of 5 patients were confirmed by positive responses on TDI-bronchoprovocation tests with clear history of work-related symptoms. For non-TDI asthma donor, 5 patients with allergic asthma sensitive house dust mites were enrolled.

To evaluate the effect of pro-inflammatory cytokine, cells ( $5 \times 10^4$  per each well) were cultured with PBMC supernatant. PBMCs were purified using Ficoll-Paque (Pharmacia, Sweden) from five TDI- and five non-TDI asthma subjects and they ( $2 \times 10^6$  cells) were incubated for 48 hr with  $10 \mu\text{g/mL}$  of TDI-HSA conjugate, which was an optimal concentration for stimulating T Cell in the previous experiment (9). Then, the supernatants were added to the epithelial cell culture. To evaluate the effect of pro-inflammatory cytokine, IL- $1\beta$  and TNF- $\alpha$  antibodies to IL- $1\beta$  and TNF- $\alpha$  (anti-IL- $1\beta$  alone, anti-IL- $1\beta$ /TNF $\alpha$ , and anti-TNF $\alpha$  alone,  $1 \mu\text{g/mL}$  each, R&D, U.S.A.) were pre-incubated with PBMC

of two groups of the patients for 24 hr, and add to the epithelial cell culture. For inhibition study by dexamethasone, four concentrations (0, 0.5, 5, 50,  $500 \mu\text{g/mL}$ ) of dexamethasone (Daewon Pharmaceutical Co., Korea) were pre-incubated for 24 hr with PBMC supernatant, and the same step was repeated. The suppressive effect was presented as % inhibition calculated as follows:  $(100 - [\text{IL-8 level preincubated with blocking antibody or dexamethasone} / \text{IL-8 level preincubated without blocking antibody or dexamethasone}]) \times 100$ . The IL-8 level was quantified using a homemade sandwich-type ELISA as described in our previous report (10). This ELISA had less than 10% of intra- and inter-assay coefficient variation.

**Statistical analysis**

The Mann-Whitney U was applied to evaluate the statistical differences between the data: A *p*-value of less than 0.05 was regarded as significant.

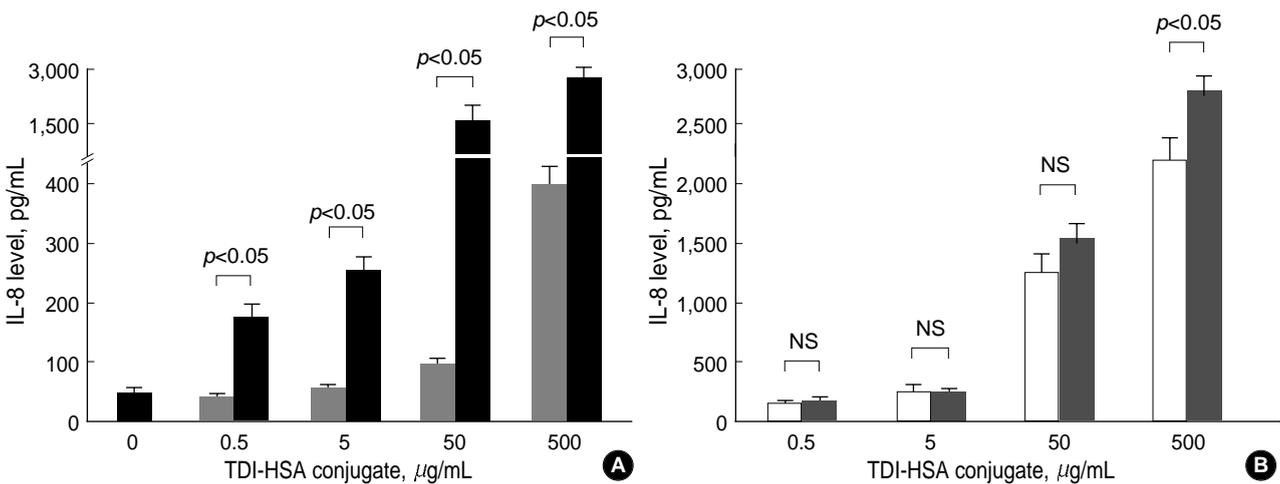


Fig. 1. TDI-induced IL-8 production from bronchial epithelial cells, Beas-2B without (■) or with (■) PBMC supernatant (A). They were compared between two different PBMC donors: TDI-induced asthma (■) and allergic non-TDI asthma subjects (□) (B). All values are presented as mean  $\pm$  S.E.

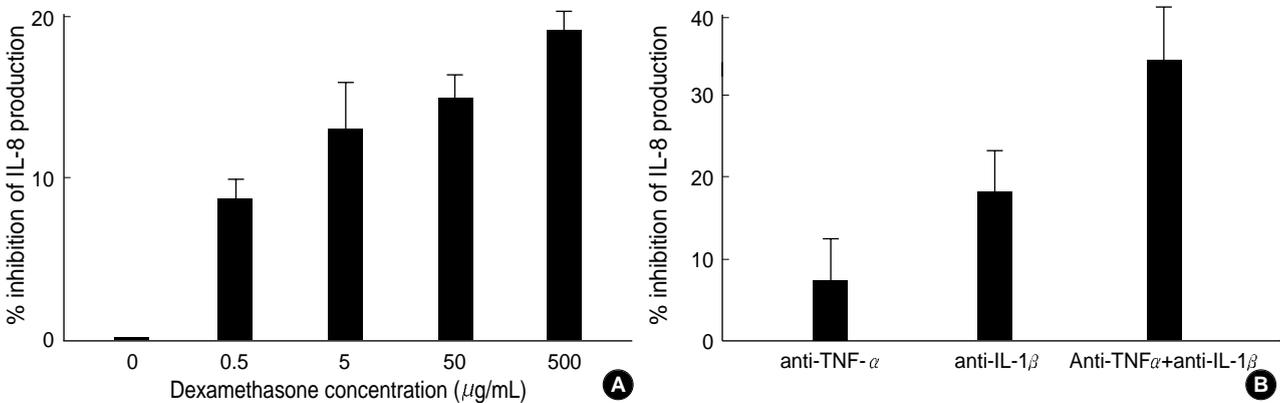


Fig. 2. Percent inhibition of IL-8 production from bronchial epithelial cells with addition of dexamethasone (A), and anti-TNF- $\alpha$  and anti-IL- $1\beta$  (B).

## RESULTS

### Production of IL-8 from bronchial epithelial cells and effect of peripheral blood mononuclear cell supernatant

IL-8 production was minimal without addition of TDI-HSA conjugate. However, the IL-8 production was increased with additions of TDI-HSA conjugate. When PBMC supernatant stimulated with TDI-HSA conjugate was added to the epithelial cell culture, the IL-8 production was increased in a dose-dependent manner. When compared with controls, IL-8 production was significantly augmented with the addition of PBMC supernatant from TDI-induced asthma subjects at each concentration of TDI-HSA conjugate ( $p < 0.05$ , respectively, Fig. 1A). When IL-8 production was compared according to two groups of PBMC donors, it tended to be higher in the TDI asthma than in non-TDI asthma, and statistical significance was noted at 500  $\mu\text{g}/\text{mL}$  of TDI-HSA conjugate incubation (Fig. 1B).

### Effect of pro-inflammation cytokines and dexamethasone

Pre-incubation of anti-IL-1 $\beta$ , and anti-IL-1 $\beta$ /TNF $\alpha$  could suppress IL-8 productions, while less inhibitions were noted with anti-TNF $\alpha$  as shown in Fig. 2A. Dexamethasone was able to suppress the IL-8 production in a dose-dependent manner (Fig. 2B).

## DISCUSSION

This study demonstrated that IL-8 production was noted with TDI-HSA incubation; however, it could stimulate PBMC to release pro-inflammatory cytokines such as IL-1 $\beta$ , synergy with anti-TNF- $\alpha$  which could augment IL-8 production from bronchial epithelial cells. IL-8 production was increased more with additions of PBMC supernatant derived from TDI-asthma subjects than in those from non-TDI asthma. The mechanism that causes TDI to release cytokines directly from epithelial cells is remains to be further investigated. It was suggested that diisocyanates bind to airway epithelial cell proteins, and are taken up by epithelial cells, which could lead to airway inflammation composing of cytokine production and cellular activation (11).

Moreover, the mechanisms how TDI could induce pro-inflammatory cytokine from PBMC, particularly in TDI-sensitized patients can not be explained in this study. Some reports showed evidence indicating activation of lymphocyte and macrophage to induce cytokine production in response to TDI-HSA conjugate (9). Our previous study (9) demonstrated that TDI-HSA conjugate activated T cell to produce cytokine production including IFN- $\gamma$  and IL-4, although

we could not try to detect other proinflammatory cytokines from them. Chemokine productions including MCP-1 and TNF $\alpha$  from PBMC from isocyanate induced asthma patients was significantly higher than those of asymptomatic exposed workers (12). Further studies will be needed to investigate how TDI stimulates pro-inflammatory cytokines from PBMC, which may augment chemokine productions from bronchial epithelial cells. This study also demonstrated incomplete inhibition of IL-8 production by dexamethasone suggesting that further mechanisms other than inhibition of NF $\kappa$ B mediate pathway via steroid (13), may be involved in the regulation of IL-8 production from epithelial cells stimulated by TDI.

In conclusion, IL-8 production induced by TDI-HSA conjugate from bronchial epithelial cells may contribute to neutrophil recruitment into the airway mucosa when exposed to TDI, which is augmented by pro-inflammatory cytokines from PBMC of TDI-induced asthma patients.

## REFERENCES

1. Park HS, Cho SH, Hong CS, Kim YY. *Isocyanate-induced occupational asthma in far-east Asia: pathogenesis to prognosis. Clin Exp Allergy* 2002; 32: s198-204.
2. Bentley AM, Maestrelli P, Saetta M, Fabbri LM, Robinson DS, Bradley BL, Kay AB. *Activated T-lymphocytes and eosinophils in the bronchial mucosa in isocyanate-induced asthma. J Allergy Clin Immunol* 1992; 89: 821-9.
3. Park HS, Hwang SC, Nahm DH, Yim HE. *Immunohistochemical characterization of the cellular infiltrate in airway mucosa of toluene diisocyanate (TDI)-induced asthma: comparison with allergic asthma. J Korean Med Sci* 1998; 13: 21-6.
4. Park HS, Jung KS, Kim HY, Nahm DH, Kang KR. *Neutrophil activation following TDI bronchial challenge to airway secretion from TDI-induced asthma. Clin Exp Allergy* 1999; 29: 1395-401.
5. Olszyna DP, Opal SM, Prins JM, Horn DL, Speelman P, van Deventer SJ, van der Poll T. *Chemotactic activity of CXC chemokines interleukin-8, growth-related oncogene-alpha, and epithelial cell-derived neutrophil-activating protein-78 in urine of patients with urosepsis. J Infect Dis* 2000; 182: 1731-7.
6. Davies DE. *The bronchial epithelium: translating gene and environment interactions in asthma. Curr Opin Allergy Clin Immunol* 2001; 1: 67-71.
7. Matsumoto K, Hashimoto S, Gon Y, Nakayama T, Horie T. *Proinflammatory cytokine-induced and chemical mediator-induced IL-8 expression in human bronchial epithelial cells through p38 mitogen-activated protein kinase-dependent pathway. J Allergy Clin Immunol* 1998; 101: 825-31.
8. Park HS, Kim HY, Nahm DH, Son JW, Kim YY. *Specific IgG, but not specific IgE, antibodies to toluene diisocyanate-human serum albumin conjugate are associated with toluene diisocyanate bronchoprovocation test results. J Allergy Clin Immunol* 1999; 104: 847-51.

9. Lee M, Park S, Park HS, Yoon JK. Cytokine-secretion patterns of T cells responding to heat-denatured-human serum albumin in toluene diisocyanate (TDI)-induced asthma patients. *J Korean Med Sci* 1998; 13: 459-65.
10. Park HS, Suh JH, Kim SS, Kwon OJ. Grain dust mite induces IL-8 production from bronchial epithelial cells: effect on neutrophil recruitment. *Ann Allergy Asthma Immunol* 2000; 84: 623-7.
11. Wisniewski AV, Lemus R, Karol MH, Redlich CA. Isocyanate-conjugated human lung epithelial cell proteins: A link between exposure and asthma? *J Allergy Clin Immunol* 1999; 104: 341-7.
12. Lummus ZL, Alam R, Bernstein JA, Bernstein DI. Diisocyanate antigen-enhanced production of monocyte chemoattractant protein-1, IL-8, and tumor necrosis factor- $\alpha$  by peripheral mononuclear cells of workers with occupational asthma. *J Allergy Clin Immunol* 1998; 102: 265-74.
13. Franklin HE, Peter JB, Michael K. Nuclear factor B: A pivotal transcription factor in chronic inflammatory disease. *N Engl J Med* 1997; 10: 1066-71.