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Immunohistochemical Characterization of the Cellular Infiltrate in Airway Mucosa of Toluene Diisocyanate (TDI)-Induced Asthma: Comparison with Allergic Asthma

Toluene diisocyanate (TDI) is the most prevalent agent in occupational asthma (OA) in Korea. The immuno-pathologic mechanism for TDI-induced bronchoconstriction remains to be clarified. We studied the immunohistochemical finding of inflammatory cells in bronchial mucosa in subjects with TDI-induced asthma. Fiberoptic bronchial biopsy specimens were obtained from nine subjects with TDI-induced asthma. Six allergic asthma sensitive to house dust mite were enrolled as controls. Bronchial biopsy specimens were examined by immunohistology with a panel of monoclonal antibodies to mast cell tryptase (AA1), secretary form of eosinophil cationic protein (EG2), pan T-lymphocyte (CD3) and neutrophil elastase (NE). There was a significant increase in the number of AA1+, EG2+ and NE+ cells in TDI-induced asthma compared to those of allergic asthma ($p=0.02$, $p=0.04$, $p=0.03$, respectively). No significant differences were observed in the number of CD3+ cells ($p=0.27$). These findings support the view that neutrophil recruitment together with eosinophil and mast cell, may contribute to the bronchoconstriction induced by TDI.

Key Words : *TDI-induced asthma, Neutrophil, Mast cell, Eosinophil, Bronchial biopsy*

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INTRODUCTION

Isocyanates are low-molecular-weight chemicals used in the manufacture of polyurethane foams, varnishes, paints, and plastics. These chemicals are currently the most common cause of occupational asthma in Korea (1) as well as in North America (2). Occupational asthma has been reported among workers exposed to toluene diisocyanate (TDI), methylene diphenyldiisocyanate (MDI), and hexa-methylene diisocyanate (HDI).

The pathogenesis of TDI-induced asthma still remains to be further clarified. Several groups of investigators have identified serum specific IgE antibodies in a part of sensitized workers (3-6). There have been a few immunohistochemical studies in bronchial mucosa of isocyanate-induced asthma subjects, which showed an involvement of inflammatory cells, such as mast cell, lymphocytes, eosinophils similarly to those of allergic asthma (7-10), and also no difference was found between two groups (7). However, there has been a report suggesting an involvement of neutrophil in TDI-induced asthma, especially in subjects with late asthmatic response (11).

In this study, in order to improve understanding the pattern of individual inflammatory cellular infiltrate in airway mucosa of TDI-induced asthma, we performed a quantitative analysis of bronchial biopsies using markers of neutrophil, mast cell, T-lymphocyte and activated eosinophil, and the results were compared to those of allergic asthma sensitive to the house dust mite.

METHODS

Subjects

Nine subjects with TDI-induced asthma and six subjects with allergic asthma who had never been exposed to any kinds of isocyanate chemicals were enrolled for this study. Their clinical characteristics were summarized in Table 1. Two subjects among TDI-induced asthma were defined as atopics, as determined by a positive skin test to at least one common allergen extract, but their symptoms were not related to exposure to such allergens. They had stopped to use inhaled corticosteroid eight

Table 1. Clinical features of the study subjects with toluene diisocyanate (TDI)-induced asthma and allergic asthma as controls

Patient	Sex/Age	Atopy	Exposure period (year)	Methacholine PC20 (mg/ml)	BPT Response	Serum specific IgE antibody*
Group I: TDI-induced asthma						
Naj	M/42	−	15	1.21	Early	+
Chk	F/35	−	5	2.4	Early	−
HuY	M/48	−	10	0.62	Late only	−
Lel	F/60	+	15	>25	Dual	−
LeJ	F/42	−	10	2.0	Dual	−
SiO	F/38	−	10	1.2	Dual	−
KiS	F/42	+	7	0.28	Atypical	+
KiY	F/41	−	5	9.5	Early	−
SiJ	F/50	−	8	4.8	Early	+
Group II: Allergic asthma						
PK	M/27	+	0	2.0	ND	+
YS	F/32	+	0	2.3	ND	+
PS	M/28	+	0	2.5	ND	+
LH	M/31	+	0	5.0	ND	+
CB	M/25	+	0	10	ND	+
KC	M/32	+	0	4.8	ND	+

BPT: TDI-bronchoprovocation test result, +: Present, −: Absent
*: Group I: Specific IgE to TDI-HSA conjugate, Group II: Specific IgE to house dust mite
ND: Not done

weeks before the study period and had been treated with inhaled/oral bronchodilators whenever symptoms developed.

All subjects underwent an interview, chest radiography, ECG, skin prick test with common allergen extracts, lung function measurement, and inhalation challenge with both methacholine and TDI (Aldrich, USA). The methacholine bronchial challenge test was done according to the method described previously (12, 13). Briefly, aerosols were generated by a DeVilbiss 646 nebulizer connected to a DeVilbiss dosimeter driven by compressed air (DeVilbiss Co. PA. USA). Five inhalations at 5-minute intervals were taken for normal saline and for each successively doubled dose of methacholine (0.075 to 25 mg/ml) until a 20% fall in FEV_1 from the post-saline value was observed. FEV_1 was measured 5 minutes after the beginning of each set of inhalations of aerosolized methacholine. The methacholine PC_{20} level was determined by interpolation from the dose-response curve. TDI bronchial challenge test was performed according to the previously described method (14). Briefly, the subjects were exposed to TDI (80:20=2,4 form : 2,6 form, Aldrich, USA) through tidal breathing in a small closed room for 5 to 15 minutes until asthmatic symptoms were induced. The concentration of TDI measured by TLD-1, a toxic gas detector with Cheakey(HDA, Scientific, USA), was 20 ppb. FEV1 and $FEF_{25-75\%}$ were measured with a spirometer (MultiSPIRO SX/PC, USA) immediately before and every hour for 8 hours after the exposure.

Bronchoscopy and bronchial biopsies

A fiberoptic bronchoscopy was performed after the bronchial challenge with TDI as described elsewhere (15). At least two endobronchial biopsies were taken through a bronchoscope (Olympus BF, type 1T 10; Olympus Co., Tokyo, Japan) with sterile forceps (FB 15C; Olympus Co.) from the bronchial mucosa of the right lower lobar bronchus. The biopsy specimen was fixed in 4% formaldehyde/1% gluteraldehyde in 0.1 M phosphate-buffered saline (PBS), then dehydrated and embedded in paraffin for immunohistology.

Immunohistochemistry and quantitation

The sections (4 μm thick) were obtained from biopsy specimens using microtome (Shandon, HM340E). Inflammatory cells were assessed by immunohistochemistry on paraffin-embedded sections using a panel of monoclonal antibodies: anti-CD3 (Dako, Denmark), antibody to secretary form of eosinophil cationic protein (EG2, Pharmacia, Sweden), anti-neutrophil elastase (NE, Dako, Denmark), and antibody to mast cell tryptase (AA1, Southampton University UK). The mouse monoclonal antibody to mast cell tryptase, kindly provided by Andrew Walls in Southampton University, have been previously characterized for their specificity and reactivity (16). Biotin conjugated rabbit anti-mouse antibodies (1:200 in TBS, Sigma, USA) were applied as the sec-

ondary antibodies and detected with the streptavidin-biotin complex using LSAB kit (Dako, Carpinteria, CA, USA) and aminoethylcabazole (AEC) as chromogen. They were counter-stained with Mayers' hematoxylin. The numbers of positively stained cells were counted in four fields per one tissue by superimposing a grid of 100 points (intersection of crosses covering a surface area of 0.5 mm^2) under an eyepiece graticulate at magnification of ×200, and expressed as the mean number of cells per mm^2 as reported by Fokkens WJ, et al. (17). For negative controls, the mouse IgG_1 antibodies (1:200 v/v) were incubated with omission of primary antibodies. To avoid observer bias, the slides were coded before analysis and read blindly.

STATISTICAL ANALYSIS

Statistical analysis included Mann-Whitney U test using SPSS version 6.0 program (Chicago, IL). Results were considered statistically significant, if $p<0.05$.

RESULTS

Mast cells

All subjects had tryptase-containing mast cells (AA1) in both epithelium and mucosal layer as shown in Fig. 1A. When the number of AA1+ cells was compared, it was significantly higher in TDI-induced asthma than in allergic asthma as shown in Fig. 2 ($p=0.02$).

Neutrophil

Elastase-containing neutrophils were found in most subjects of TDI-induced asthma as shown in Fig. 1B, and the number of cells was significantly higher in TDI-induced asthma than in allergic asthma shown in Fig. 2 ($p=0.03$).

Eosinophil

In all the subjects, there are abundant EG2+ cells which were significantly higher in TDI-induced asthma than those of allergic asthma as shown in Fig. 2 ($p=0.04$), indicating activated eosinophil are involving in airway inflammation of TDI-induced asthma as well as in allergic asthma.

T-lymphocytes

Three subjects with TDI-induced asthma had CD_3+ T lymphocyte and their numbers varied from 0 to 26/mm^2. The median and range of the numbers of positive cells in two groups are shown in Fig. 1. There was no significant difference in CD_3+ cell counts between two groups ($p=0.27$).

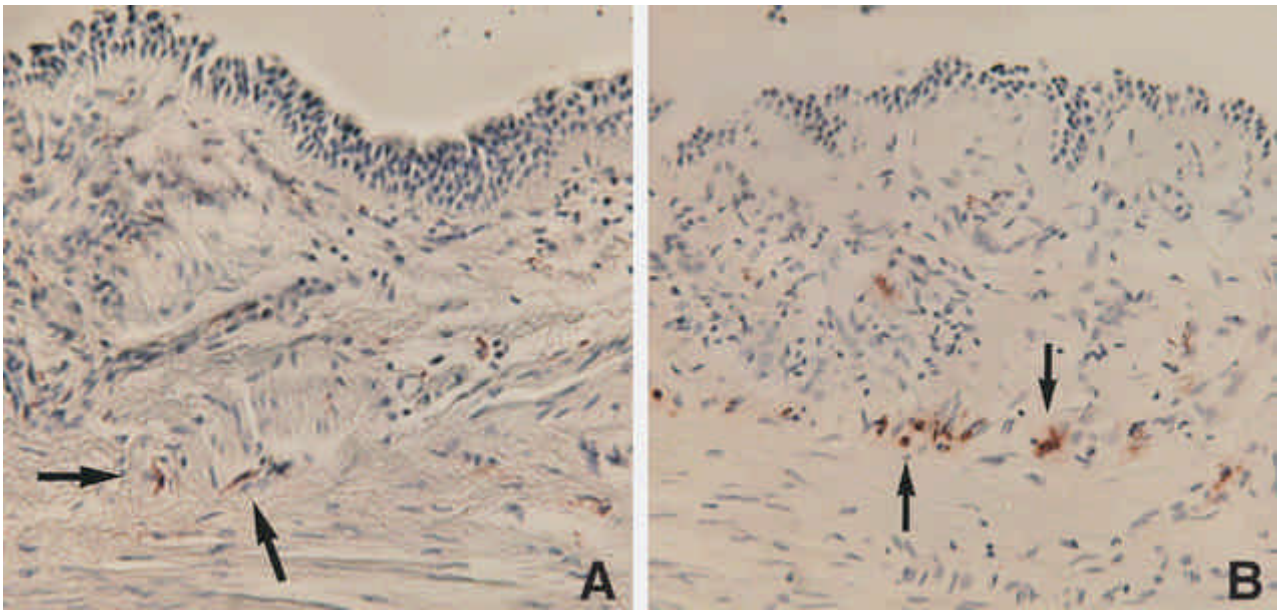

Fig. 1. Infiltrated cells of bronchial mucosa section of TDI-induced asthma positively stained with antibodies to mast cell tryptase (A, ×200) and neutrophil elastase (B, ×200).

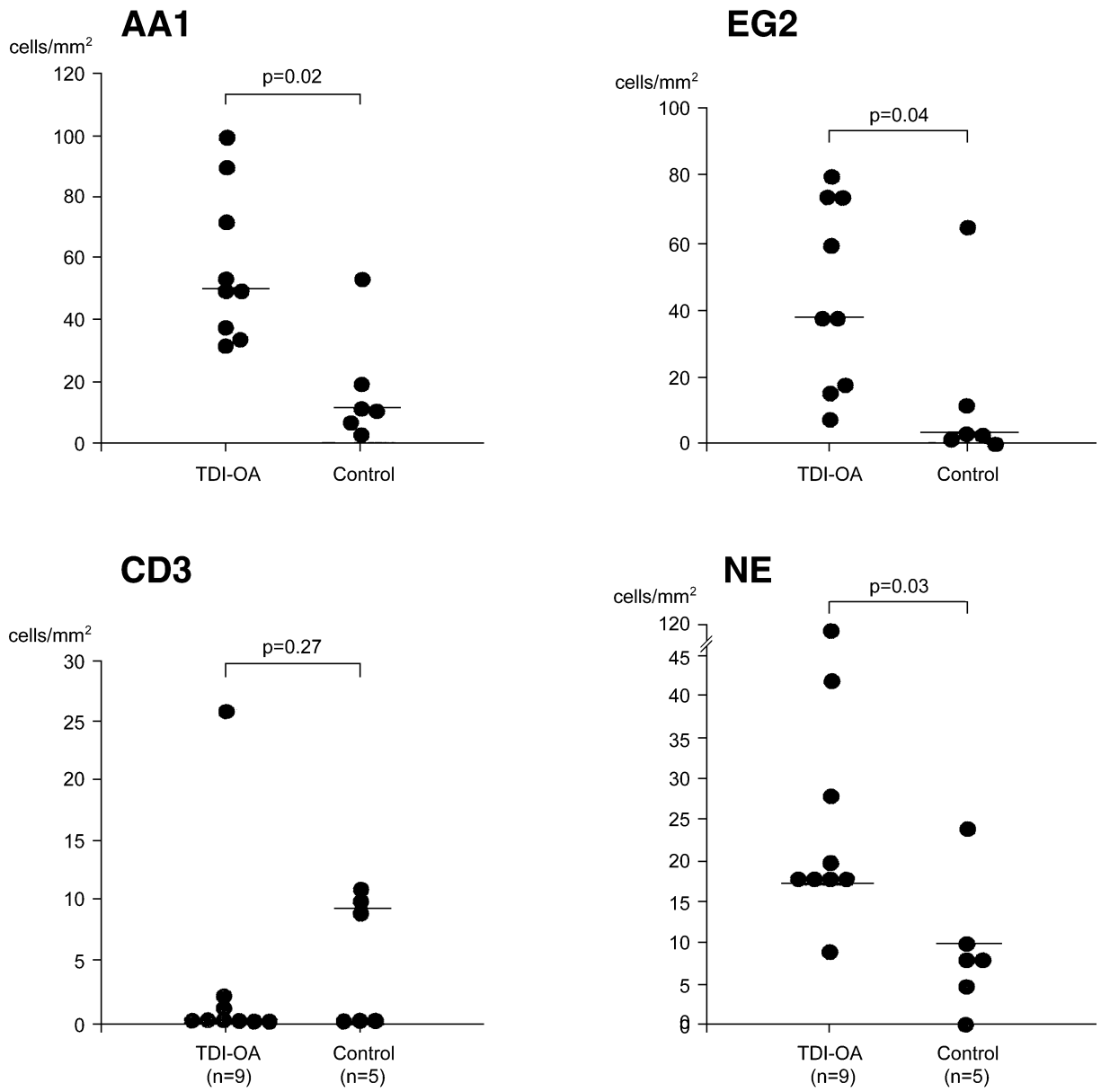

Fig. 2. Comparison of inflammatory cell counts expressing AA1 (mast cell), EG2 (eosinophil), CD3 (pan T cell) and NE (neutrophil) in bronchial mucosa section of subjects with TDI-induced asthma (TDI-OA) and allergic asthma (control).

DISCUSSION

Airway inflammation is an important characteristic of asthma. Fiberoptic bronchial biopsy findings from subjects with allergic asthma have shown epithelial and submucosal inflammatory changes, and the degree of inflammatory response correlated with the level of airway responsiveness. Numerous studies have identified increased numbers of epithelial cells and mast cells in bronchoalveolar lavage (BAL) fluid of subjects with mild asthma which correlate with the degree of airway responsiveness (18-22). In this study, we demonstrated that there was an inflammatory change, composed of tryptase-secreting mast cells, neutrophils, T lymphocytes, and actively secreting eosinophils in the bronchial mucosa of TDI-induced asthma as well as in allergic asthma.

TDI is the most prevalent cause of occupational asthma in Korea (1, 6) as well as in westernized countries (2). The pathogenetic mechanism is still unclear, but it has been suggested that TDI may act as a hapten, combining with protein-carrier molecules to provoke an immune response (5, 6, 23). Elevated serum-specific IgE antibodies have been detected in a part of subjects with TDI-induced asthma, which may reflect the involvement

of other immunologic and/or non-immunologic mechanisms. In the aspects of cellular immunology, the possible role of T-lymphocyte in isocyanate-induced asthma has been suggested because low-molecular-weight haptens may possibly be recognized by T cells in the same way as an allergen in atopic asthma (7, 15). In the present study, there were significantly higher numbers of inflammatory cells such as mast cells, eosinophils and neutrophils in bronchial mucosa of subjects with TDI-induced asthma, without regard to their serum specific IgE antibody to TDI-HSA conjugate. Most of occupational asthma had airway hyperresponsiveness to methacholine (2). However, in case of isocyanate-induced asthma, an appreciable number of TDI-induced asthma subjects had a negative result on the initial methacholine bronchial challenge test, then developed airway hyperresponsiveness to methacholine after the TDI-bronchoprovocation test (13, 24). In this study, one of the TDI-induced asthma subject had a negative result on the initial methoacholine bronchial challenge test. These findings confirmed that a negative result at initial methoacholine bronchial challenge test did not preclude TDI-induced occupational asthma.

There has been one study suggesting an involvement of neutrophil in pathogenesis of isocyanate-induced asthma, especially in those with late asthmatic response (11), in keeping with the study of grain dust-induced occupational asthma, in which neutrophil counts in bronchial mucosa was significantly higher than those from allergic asthma (25). Significant neutrophilia (up to 40%) was observed in BAL fluid of the subjects with grain dust-induced occupational asthma (26). Furthermore, a few investigators reported a significant increase of serum neutrophil chemotactic activity from the subjects with isocyanate-induced asthma. The origin of neutrophil chemotactic activity in TDI-induced asthma was speculated to be mast cells or basophils (27). Recently, IL-8 has been discovered to be an inflammatory cytokine that is a potent activating and chemotactic factor of neutrophil (28, 29) in respiratory mucosa. IL-8 is abundant in bronchial epithelial cell, eosinophil, monocyte, fibroblast and endothelial cell (30). Moreover, mast cell could release IL-4, TNFα, IL-8, leukotrienes and platelet activating factor which could induce neutrophil chemotaxis (31). Our recent investigation on cytokines in the induced sputum of TDI-induced asthma revealed that IL-8 was abundant in the sputum and, significantly increased after the TDI bronchial challenges, which suggested that IL-8 might participate in neutrophil recruitment to bronchial mucosa of TDI-induced asthma (unpublished data). In the present study, both mast cell and neutrophil counts were significantly higher in TDI-induced asthma than in allergic asthma. There was a significant correlation between NE+ and AA1+ cells ($r=0.73$, $p=0.02$, data was not shown). Therefore, mast cells found in bronchial mucosa of TDI-induced asthma might contribute to the neutrophil recruitment into airway mucosa. Further investigations will be needed to elucidate these mechanisms.

T cells, especially Th2 cells are central regulatory cell in airway inflammation of asthma patients whether they were extrinsic or intrinsic (18). A previous study also demonstrated increased infiltration of activated T cell in bronchial mucosa of isocyanate-asthma similarly to extrinsic asthma (7). Release of IFN-γ from peripheral CD8+ T cell was reported when exposed to TDI (32). In the present study, some subjects with TDI-induced asthma had CD3+ T cells in bronchial mucosa. Further investigations will be needed to understand the role of T cell in TDI-induced asthma.

In conclusion, this study supports the view that two effector cells, mast cell and neutrophil in harmony with eosinophil and T cell may be placed in the center of airway inflammation induced by TDI.

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REFERENCES

1. Park HS. *Occupational asthma. Korean J Ind Med 1995; 34: 80- 92.*
2. Chan-Yeung M. *Occupational asthma. Chest 1990; 98: 148S-61S.*
3. Karol MH, Loset HH, Alarie YC. *Tolyl-specific IgE antibodies in workers with hypersensitivity to toluene diisocyanate. Am Ind Hyg Assoc J 1978; 39: 454-8.*
4. Danks JM, Cromwell O, Buckingham JA, Newman Taylor, Davies RJ. *Toluene diisocyanate induced asthma: evaluation of antibodies in the serum of affected workers against a tolyl mono-isocyanate protein conjugate. Clin Allergy 1981; 11: 161-8.*
5. Pezzini A, Riviera A, Paggiaro P, Spiazzi A, Gerosa F, Filieri M, Toma G, Tridente G. *Specific IgE antibodies in twenty eight workers with diisocyanate-induced bronchial asthma. Clin Allergy 1984; 14: 453-61.*
6. Park HS, Nahm DH. *Isocyanate-induced occupational asthma: challenge and immunologic studies. J Korean Med Sci 1996; 11: 314-8.*
7. Bentley AM, Maestrelli P, Saetta M, Fabbri LM, Robinson DS, Bradley BL, Jeffery PK, Durham SR, Kay AB. *Activated T-lymphocytes and eosinophils in the bronchial mucosa in*

isocyanate-induced asthma. J Allergy Clin Immunol 1992; 89: 821-8.

8. Saetta M, Di Stefano A, Maestrelli P, De Marzo N, Milani GF, Pivirotto F, Mapp CE, Fabbri LM. *Airway mucosa inflammation in occupational asthma induced by toluene diisocyanate. Am Rev Respir Dis 1992; 145: 160-8.*
9. Saetta, M, Maestrelli P, Di Stefano A, De Marzo N, Milani GF, Pivirotto F, Mapp CE, Fabbri LM. *Effect of cessation of exposure to toluene diisocyanate (TDI) on bronchial mucosa of subjects with TDI-induced asthma. Am Rev Respir Dis 1992; 145: 169-74.*
10. Di Stefano A, Saetta M, Maestrelli P, Milani GF, Pivirotto F, Mapp CE, Fabbri LM. *Mast cells in the airway mucosa and rapid development of occupational asthma induced by toluene diisocyanate. Am Rev Respir Dis 1993; 147: 1005-9.*
11. Fabbri LM, Boschetto P, Zocca E, Milani G, Picicotto F, Plepari M, Burlina A, Licata B, Mapp CE. *Bronchoalveolar neutrophilia during late asthmatic reactions induced by toluene diisocyanate. Am Rev Respir Dis 1987; 136: 36-42.*
12. Chai H, Farr RS, Frehlich LA, Mathison DA, McLean JA Rosenthal RR. *Standardization of bronchial inhalation challenge procedure. J Allergy Clin Immunol 1975; 56: 323-7.*
13. Park HS, Park JN, Kim JW, Kim SK. *Clinical and immunological evaluation of isocyanate-exposed workers. J Korean Med Sci 1992; 7: 122-7.*
14. Park HS, Nahm DH. *Prognostic factors for toluene diisocyanate-induced occupational asthma after the removal from exposure. Clin Exp Allergy 1997; 27: 1145-50.*
15. Maestrelli P, Stefano AD, Occari P, Turato G, Milani G, Pivirotto F, Mapp CE, Fabbri LM, Saetta M. *Cytokines in the airway mucosa of subjects with asthma induced by toluene diisocyanate. Am J Respir Crit Care Med 1995; 151: 607-12.*
16. Bradding P, Feather LH, Wilson S, Bardin PG, Heusser CH, Hogate ST, Howarth PH. *Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. J Immunol 1993; 151: 3853-65.*
17. Fokkens WJ, Holm AF, Rijntjes E, Mudler PGH, Vroom TM. *Characterization and quantitation of cellular infiltrates in nasal mucosa of patients with grass pollen allergy, non-allergic patients with nasal polyps and controls. Int Arch Allergy appl Immunol 1990; 93: 66-72.*
18. Kay AB. *Asthma and inflammation. J Allergy Clin Immunol 1991; 87: 893-910.*
19. Wardlaw AJ, Dunnette S, Gleich J, Collins JV, Kay AB. *Eosinophils and mast cells in bronchoalveolar lavage in subjects with asthma. Am Rev Respir Dis 1988; 137: 62-9.*
20. Kirby JG, Hargreave FE, Gleich GJ, O'Byne PM. *Bronchoalveolar cell profiles of asthmatic and non-asthmatic subjects. Am Rev Respir Dis 1987; 136: 379-83.*
21. Flint KC, Leung KB, Hudspith BN, Brostoff J, Pierce FL, Johnson NM. *Bronchoalveolar mast cells in extrinsic asthma: a mechanism for the initiation of antigen-specific bronchoconstriction. Br Med J Clin Res Ed 1985; 291: 923-6.*
22. Tomioka M, Ida S, Shinda Y, Ishihara T, Takishima T. *Mast cells in bronchoalveolar lumen of patients with bronchial asthma. Am Rev Respir Dis 1984; 129: 1000-5.*
23. Baur X, Dewier M, Fruhmann G. *Detection of immunologically sensitized isocyanate workers by RAST and intracutaneous skin tests. J Allergy Clin Immunol 1984; 73: 610-8.*
24. Park HS, Cho YS, Park JN, Baik JH, Rhu NS, Cho DI, Kim JW. *Significant changes if bronchial hyperresponsiveness to methacholine after early asthmatic reaction to TDI in a TDI-sensitive asthmatic worker. J Korean Med Sci 1990; 5: 185-8.*
25. Park HS, Jung KS, Hwang SC, Nahm DH, Yim H. *Neutrophil infiltration and release of IL-8 in airway mucosa from subjects with grain dust-induced occupational asthma. Clin Exp Allergy 1998 (in press).*
26. Jung KS, Park HS. *Bronchoalveolar lavage finding and neutrophil chemotactic activity in grain dust-induced occupational asthma. Kor J Tuber Respir 1996 (abstract).*
27. Sastre J, Banks DE, Lopez M, Barkman HW, Salvaggio JE. *Neutrophil chemotactic activity in toluene diisocyanate (TDI)-induced asthma. J Allergy Clin Immunol 1990; 85: 567-72.*
28. Mukaida N, Shiroo N, Matsushima K. *Genomic structure of the human monocyte-derived neutrophil chemotactic factor IL-8. J Immunol 1989; 143: 1366-71.*
29. Yuo A, Kitagawa S, Kasahara T, Matsushima K, Saito M, Takaku F. *Stimulation and priming of human neutrophils by interleukin-8: cooperation with tumor necrosis factor and colony-stimulating factors. Blood 1991; 78: 2708-14.*
30. Teran LM, Davies DE. *The chemokines: their potential role in allergic inflammation. Clin Exp Allergy 1996; 26: 1005-19.*
31. Redington AE, Howarth PE. *Mast cells, cytokines and asthma. Can Respir J 1994; 1: 1-10.*
32. Mastrelli O, Del Prette GF, De Carli Map CE, Fabbri LM. *Activated CD8 T-lymphocytes producing interferon-gamma and interleukin-5 in bronchial mucosa of subjects sensitized to toluene diisocyanate. J Allergy Clin Immunol 1993; 91: 220 (abstract).*