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Occupational Asthma and IgE Sensitization to Grain Dust

To evaluate type I hypersensitivity to grain dust (GD), its prevalence and relationship to respiratory dysfunction, we studied clinical and immunologic features, including skin prick tests (SPT), serum specific IgE, and bronchoprovocation tests of 43 employees working in the animal feed industry. To further characterize IgE-mediated reaction, SDS-PAGE and electroblot studies were performed. Our survey revealed that 15 (34.9%) subjects had work-related skin response ($\geq$2+ of A/H ratio) to GD, thirteen (30.2%) had high specific IgE antibody against GD. The specific IgE antibody was detected more frequently in symptomatic workers (40%) than in asymptomatic workers (11%). Significant association was found between specific IgE antibody and atopy or smoking ($p<0.05$). The ELISA inhibition test of GD revealed significant inhibitions by GD extract and minimal inhibitions by the house dust mite, storage mite and corn dust. Immunoblot analysis showed 8 IgE binding components within GD ranging from 13.5 to 142.5 kDa. Two bands (13.5, 33 kDa) were bound to the IgE from more than 50% of the 14 sera tested. In conclusion, these findings suggest that GD inhalation could induce IgE-mediated bronchoconstriction in exposed workers.

Key Words : *Occupational diseases, asthma; Asthma; Dust grain dust; IgE; Immunoblotting*

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INTRODUCTION

Chronic inhalation of GD has been shown to cause acute and chronic airway injury characterized by bronchitis and airflow obstruction (1, 2). The level of airborne GD is related to the presence of bronchial hyperreactivity, as well as to the development of airflow limitation across a work shift (3, 4). In aspect of pathogenic mechanism of GD-induced bronchoconstriction, there have been no report suggesting an IgE-mediate mechanism in GD-induced occupational asthma. In order to evaluate type I hypersensitivity to GD and its relationship to respiratory dysfunction, we performed SPT and ELISA to detect specific IgE antibody to GD in 43 exposed workers of the animal feed industry. We also performed methacholine bronchial challenge tests and bronchoprovocation tests with GD in symptomatic workers to confirm bronchial sensitization. Furthermore, IgE-binding components were detected using immunoblot analysis. ELISA-inhibition tests were performed to investigate the binding specificity of IgE antibody, and to exclude the contamination of other common inhalant allergens.

MATERIAL AND METHODS

Subjects

All of the 43 subjects exposed to GD composed of corn, rye, wheat, and barley, were male and worked for the Dongbang feed industry in Suwon, Korea. Of these employees, 31 were process workers who mixed the materials as well as carried them. They were classified as group II (intermediate exposure) and group III (high exposure) according to exposure intensity which was measured by a dust air sampler (Gillian INS, USA). Twelve workers were office workers and were classified as group I (low exposure group). Sera from 43 workers were collected and stored at -20℃. Twenty seven individuals who had never been exposed to GD and demonstrated negative skin tests to 50 common inhalant allergens were employed as control subjects. The subjects gave their written informed consent as regulated by Ajou University Hospital, Suwon, Korea.

Questionnaires

Initially, industrial physicians together with allergists administered a questionnaire, which was a National Heart and Lung Institute modification of the British Medical Research Council questionnaire (5), with additional questions later if needed. In evaluating and comparing these data, the following definitions were used: Lower respiratory symptoms referred to cough, sputum, chest tightness, or shortness of breath. Symptomatic workers were those who had experienced lower respiratory symptoms during and after the GD exposure.

Preparation of extracts

GD was obtained from the patient's workplace. They were extracted with phosphate-buffered saline [(PBS, pH 7.5), 1:5 w/v] at 4℃ for 1 h followed by centrifugation at 5,000 rpm. The supernatants were passed through syringe filter (MSI, USA) and used for bronchoprovocation test at a concentration of 1:10 w/v. For the skin-prick test, the 1:5 w/v extract was mixed with an equal amount of sterile glycerine. The supernatant was dialyzed (the cut-off molecular weight was 6 kDa) against 4 liters of distilled water at 4℃ for 48 h, and lyophilized at -70 ℃ for the preparation of antigens used in ELISA, ELISA inhibition and immunoblot assay.

Allergy skin test

SPT with seven common inhalant allergens (alder, rye grass, ragweed, mugwort, *Aspergillus* spp, *Dermatophagoides farinae*, cat fur, Bencard Allergy Unit, England), GD, and histamine (1 mg/ml, Bencard Allergy Unit) were performed on the volar side of both forearms simultaneously. The reactions were read 15 minutes later. The wheal and erythema size of each antigen (A) and histamine (H) was measured by maximum diameter (A1 and H1) and vertical length at the mid portion of the maximal length (A2 and H2). Skin reactivity was expressed as the area ratio of wheal size of the antigen to that of the histamine ($<A1+A2/H1+H2>^2$: A/H ratio). If A/H ratio was from 0.1 to 1 and erythema had a diameter of <21 mm, it was read as 1+. If A/H ratio was from 0.1 to 1, but erythema was >21 mm, it was read as 2+. If A/H ratio was from 1 to 2, it was read as 3+. If A/H ratio was from 2 to 3, it was read as 4+. If A/H ratio was >3, it was read as 5+. A positive responder was defined as one who demonstrated >2+ response to GD extract on SPT.

Protocol

All symptomatic subjects were admitted to the Allergy Clinic at Ajou University Hospital, and before the inhalation challenge was initiated, a standard protocol was followed in all cases. A screening evaluation, which included a complete history, physical examination, pulmonary function tests, chest X-ray, and electrocardiogram were performed on admission. A peripheral blood sample was obtained for eosinophil and leukocyte counts. Baseline forced expiratory maneuvers were performed using a MultiSPIRO-SX/PC (USA) with standard protocols.

Bronchoprovocation test with GD

Airway hyperresponsiveness (AH) to methacholine was tested according to the method described previously with some modifications (6). Bronchoprovocation tests were performed according to standard studies of occupational asthma (7). On a control day, normal saline was administered from a nebulizer 646 connected to a dosimeter (DeVilbiss Co. USA) operated at a pressure of 20 psi. The subject was asked to inhale the aerosol five times from functional residual capacity to total lung capacity, then observed for 7 hours. On a different day, GD extract was inhaled from 1:1000 to 1:10 w/v (1:1000, 1:100, 1:10) with ten min intervals. In the pulmonary function test, the functional vital capacity, the forced expiratory volume in one second (FEV_1), and the FEF_{25-75} was measured with a MultiSPIRO-SX/PC (USA) before, and 10 min after each inhalation. FEV_1 and FEF_{25-75} were measured every 10 min during the first hour, and pulmonary function tests were performed every hour for 7 hours after the challenge. A positive reaction was defined when FEV_1 decreased 20% or more from the baseline value. When asthmatic reaction was observed within 1 hour, it was called as early response. Late response was defined as an asthmatic response occurring from 4 to 24 hours after the challenge. In order to exclude the possibility of non-specific effect from GD, same procedures were performed on two house dust mite-sensitive asthmatic subjects with no exposure history to GD.

ELISA for specific IgE antibodies to grain dust extracts

The presence of specific IgE antibody to GD was determined by ELISA. Microtitre plate was first coated with GD extract (5 μg of weight per well), and left at 4℃ overnight. Each well was washed 3 times with 0.05% Tween-phosphate buffered saline (PBST), and remaining binding sites were blocked with 350 μl of 3% BSA-PBST for 1 hour. The wells were then incubated for 2 hours at room temperature with 50 μl of either the patients' or 27 control sera. After another washing step, 50 μl of the 1:1000 v/v biotin-labeled goat anti-human IgE antibody (Vector Co. USA.) were incubated

for 2 hours. The wells were then washed and incubated with 1:1000 v/v streptavidin-peroxidase (Sigma Co. USA) for 30 min, followed by incubation with ABTS (2.2'-azinobis-3-ethyl-benzthiazoline sulfuric acid in a citrate phosphate buffer) for 10 min. The reaction was stopped by the addition of 2 N sodium azide and absorbance was read at 410 nm by an automated microplate reader. All assays were performed in triplicate. Positive cut-off value (0.064) was derived from mean +2.S.D. of the absorbence value of control subjects.

Measurement of total IgE

The total IgE was measured with a DPC kit (USA) according to the manufacturer's directions.

SDS-PAGE and immunoblot analysis

GD samples (10 mg/ml) were each dissolved in a buffer (0.5 M tris-HCI, pH 6.8, 2.5 ml, glycerol 2.0 ml, 10% w/v SDS 4.0 ml, 0.1% bromophenol blue 0.5 ml and distilled water 1.0 ml) and boiled. Ten microlitres of standard marker (Novex, CA, USA) and antigen solutions were applied to a Novex precast Tris-glycine homogenous gel (14% acrylamide) to facilitate separation of GD. Electrophoresis was performed with a Novex Minicell for 90 min at a constant voltage of 125. The gel was fixed and stained with Coommassie brilliant blue. For immunoblotting, the gel was soaked in a transfer buffer (12 mM Tris, 96 mM glycine in 20% methanol) for 10 minutes. Electroblotting was carried out for 90 min at 200 mA using a Novex western transfer apparatus. The blotted polyvinylidine difluoride membrane (Novex) was then treated with a 10% BSA-Tris buffered saline solution for 1 hour to block non-specific protein binding. The patient's and control sera were diluted to 1:2 v/v with a TBS buffer (Tris 10 mM, NaCl 150 mM, 0.05% Tween-20). The membrane was incubated with the patient's and control sera for 2 hours at room temperature in a special device (Multiscreen device; Bio-Rad, Hercules, CA). It was then washed with TBS-0.1% Tween-20 Triton for 10 min, and 0.5% NaCl in TBS-T for an another 10 min. Biotin-conjugated anti-human IgE antibody (1:200, Vector, USA) was incubated with second antibody for 1 hour and washed again. The membrane was incubated with alkaline phosphatase-conjugated streptavidin (1:1000 v/v, Sigma Co.) for 1 hour. Finally, the substrate (NBT/BCIP kit, Sigma Co., USA) was applied to the membrane until positive bands appeared. After the reaction, the membrane was washed with distilled water and observed.

Statistical analysis

The χ^2 tests and ANOVA using the SPSS version 7.0 (Chicago) and Statview 512+ (Macintosh) were applied to evaluate the statistical differences between the two data. A p value of 0.05 or less was regarded as significant.

Table 1. Clinical features of the study subjects

Patient	Sex	Atopy	PC20 methacholine (mg/ml)	Peripheral eosinophil count (/μl)	Specific IgE antibody	Skin reactivity**	Total IgE level (IU/ml)	BPT response
Group A								
PC	M	P	24	1,000	A	3	4,450	Dual
JD	M	P	>25	300	P	0	2,130	Early
LB	M	P	>25	100	P	1	2,460	Early
IH	M	P	2.5	1,800	A	1	2,886	Early
YJ	M	P	>25	100	P	1	486	Early
IJ	M	P	25	300	A	5	198	Early
Group B								
KoS	M	P	>25	300	P	2	486	Negative
KiY	M	A	>25	300	P	0	657	Negative
LeH	M	P	10.3	200	A	0	170	Negative
ChB	M	P	2.2	600	A	0	518	Negative
KiC	M	P	2.3	1,400	P	0	ND	Negative
KoY	M	P	>25	300	A	0	581	Negative
Jun	M	P	>25	0	A	1	35	Negative
Jin	M	P	>25	200	P	1	260	Negative
LeJ	M	P	18	1,700	A	1	847	Negative

M: Male, P: Presence, A: Absence, ND: Not done, BPT: Bronchoprovocation test result with grain dust
* Atopy was defined as a positive reactor to more than one common inhalant allergen on skin prick test
** Skin reactivity: A/H ratio to grain dust

RESULTS

Responses to survey

On the SPT with GD, eight (18.6%) of the 43 subjects demonstrated ≥2+ of A/H ratio to GD. Fifteen (34.9%) complained of lower respiratory symptoms with or without nasal symptoms during and after GD exposure. When methacholine bronchial challenge tests were performed on 15 subjects who complained of respiratory symptoms, seven had airway hyperresponsiveness (AH) to methacholine. A positive result was defined if the subject showed a significant bronchoconstriction at ≤25 mg/ml of methacholine inhaled. Three of them showed a negative result on the initial methacholine bronchial challenge test. When bronchoprovocation tests with GD were performed on 15 symptomatic subjects, immediate asthmatic responses were noted in five workers; the other one worker demonstrated a dual response. Table 1 summarizes the clinical data of six asthmatic subjects with positive challenges (group A) and nine symptomatic subjects with negative challenges (group B). Two house dust mite-sensitive asthma also showed negative challenges

Specific IgE antibody to grain dust

The features of the specific serum IgE bindings to GD in all workers and in 27 controls are illustrated in Fig. 1. The cut-off absorbance value of positive specific IgE binding was determined as 0.064, which was derived from the mean and two standard deviations of absorbance value of controls. When ELISA was considered as positive (>0.064 of absorbance value), nine (21%) were positive, the specific IgE antibody was detected more frequently in six of 15 symptomatic subjects (40%) than

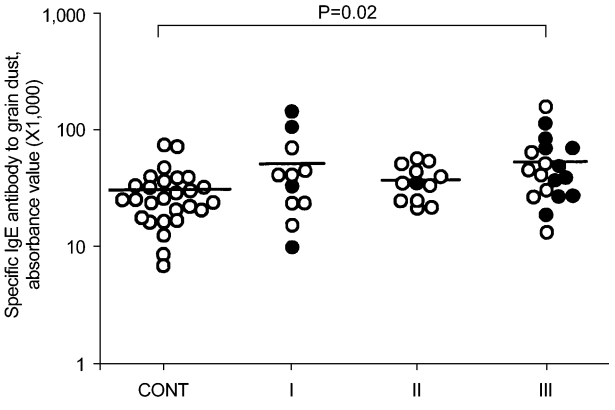

Fig. 1. Specific IgE to grain dust in control and three different exposure groups. I Low exposure, II: Intermediate exposure, III: High exposure. ●: Symptomatic subjects, ○: Asymptomatic subjects.

in asymptomatic subjects (11%, p=0.02). Three employees without respiratory symptoms demonstrated high specific IgE bindings. No difference was found according to exposure intensity within the workplace (p=0.07). Table 2 shows the relationship between the specific IgE binding and the SPT results. The prevalence of the specific IgE antibody did not increase according to skin reactivity. Among the 6 asthmatic workers, three (50%) had high specific IgE antibodies. The relationship between the specific IgE antibody and cigarette smoking is presented in Table 3. Significant associations were found between them (p<0.05). When atopy was defined as a positive reactor (A/H ratio ≥2+) to one or more allergens on SPT with 7 common inhalant allergens, significant association was found between atopy and the specific IgE binding (p<0.05, Table 3).

ELISA inhibition test

The dose-response pattern of the inhibitory effect of various concentrations of GD on the GD-ELISA inhibition test is illustrated in Fig. 2. No inhibition was noted with additions of corn dust, storage mite or *D. pteronyssinus*.

Total IgE level

A high total IgE level (≥160 IU/ml), which was derived from the mean and 2 standard deviations of the total IgE level in non-atopic patients in Korea, were found in 24 (55.8%) of those employees tested. The correlation between the specific IgE level and the expo-

Table 2. Skin reactivity and specific IgE antibody to grain dust

A/H	Number	Positive specific IgE binding (%)	Mean absorbance value (×1,000)	Range (×1,000)
0	23	5 (18)	41.1	13-112
1	11	4 (27)	64.9	10-160
2	5	2 (29)	42.4	22-69
3	2	1 (50)	48.5	33-64
5	1	1 (100)	63.0	

Table 3. Association between specific IgE antibody to grain dust and smoking and atopic status

	Specific IgE antibody to grain dust		p value
	Positive	Negative	
Current & Ex-smoker	7/30 (23.3)	23/30 (76.6)	p<0.05
Non-smoker	11/13 (84.6)	12/13 (92.3)	
Atopy	7/29 (24.1)	22/29 (75.8)	p<0.05
Non-atopy	12/14 (85.7)	2/14 (14.2)	

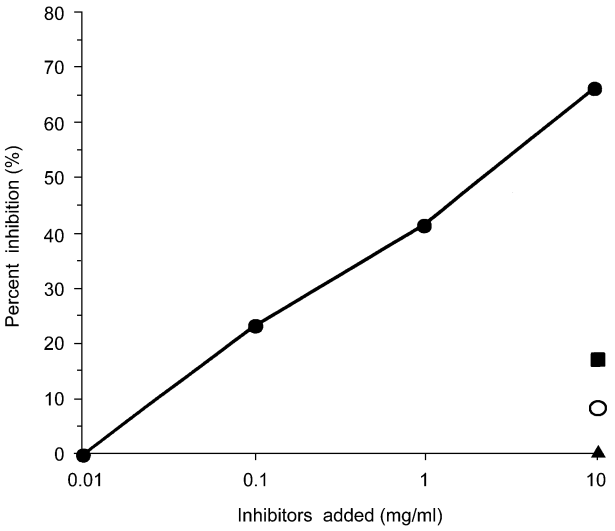

Fig. 2. Percent inhibition of grain dust-ELISA with serial addition of grain dust (●), corn dust (■), storage mite (●) and *D. pteronyssinus* (▲).

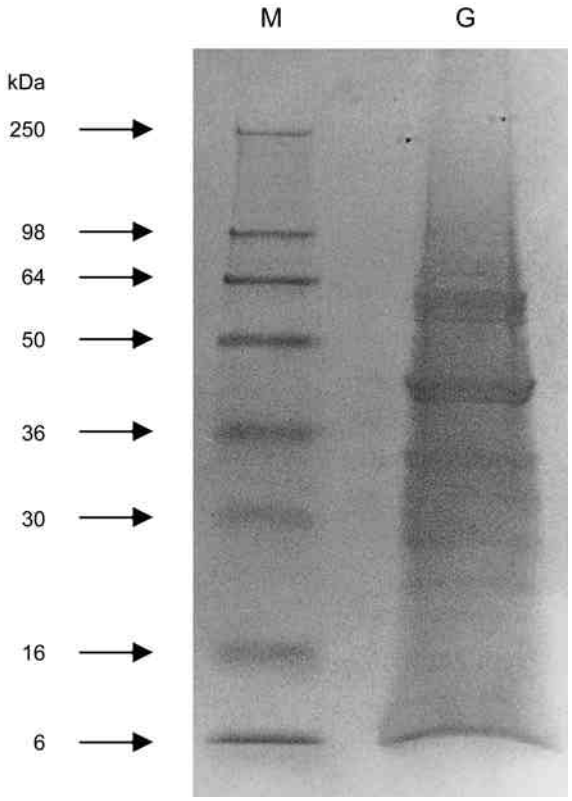

Fig. 3. 14% SDS-PAGE and protein staining of grain dust extracts.

sure duration was insignificant at r=−0.01 (data is not shown). The correlation coefficient between the total IgE level and the specific IgE was also insignificant at r=0.09 (data is not shown).

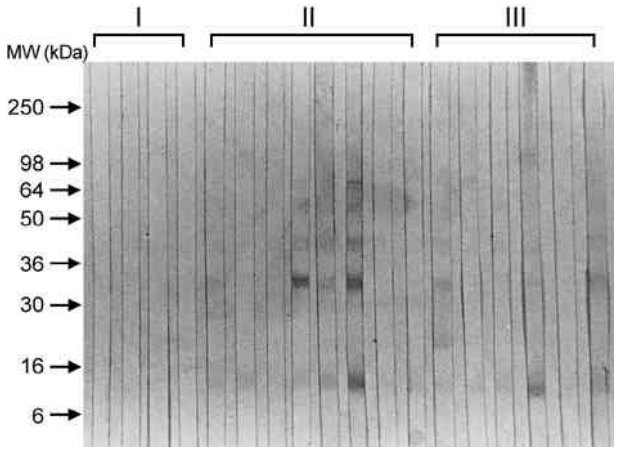

Fig. 4. Immunoblot analysis of grain dust extracts using control sera (I), symptomatic subjects with negative challenges (II) and asthmatic subjects with positive challenges (III).

Gel electrophoresis and immunoblot analysis

In order to determine the protein components of the GD extract, it was analyzed by 14% SDS-PAGE. Fig. 3 shows that the GD extract was resolved into bands ranging from 6 to 64.1 kDa. Fig. 4 shows IgE binding components in 8 of group II and 6 of group I works, as well as 4 control sera on a blotted membrane. There were 8 bands bound to IgE antibody with molecular weights ranging from 13.5 to 142.5 kDa. 13.5 kDa was the highest (86%) frequency of the IgE binding in the 14 sera tested. Two bands (13.5 kDa, 33 kDa) were bound to the IgE from 50% of the sera tested.

DISCUSSION

We demonstrated clinical and immunologic responses of workers exposed to GD. Fifteen (34.9%) of 43 subjects tested had pronounced respiratory symptoms. Among them, 13 (30.2%) subjects had specific serum IgE antibodies to GD, and seven had AH. Inhalation challenge tests with GD induced immediate asthmatic responses in five subjects, and a dual asthmatic response in 1 subject. In patients with serum specific IgE antibody and IgE-binding component, the IgE-mediated reaction is likely to be responsible for their asthmatic symptoms. In this study, three of group I workers had high specific IgE antibodies to GD. The failure to detect serum specific IgE antibody in three workers might indicate the involvement of another non-immunologic or immunology, such as IgG mediated mechanism (8), but our previous study revealed that the possibility of the specific IgG_4 as a sensitizing antibody seemed to be very low (9).

We successfully detected the specific IgE to GD by ELISA in exposed workers. ELISA inhibition test confirmed the specificity of the specific IgE binding. Moreover, immunoblot analysis showed eight IgE binding components within GD. Two major allergens were identified. Positive skin test, the presence of serum specific IgE antibody, and IgE-binding component on immunoblot finding indicated that GD acts as a sensitizing allergen when exposed. Some of the positive reactors on skin test gave negative responses in the ELISA. These discrepancies may have occurred because GD had a histamine releasing effect as suggested by the previous study (9).

Most patients with symptomatic occupational asthma have been known to have demonstrable AH (10). In this study, three patients had mild degree of AH to methacholine. Three had a negative result on initial methacholine challenge test. Furthermore, when the following methacholine challenge test was done at 24 hours after the GD-bronchoprovocation test, AH increased more than two steps in two subjects (data was not shown). These results suggest that GD inhalation could induce airway inflammation, which may contribute to progression of AH.

The striking finding of this study was that total IgE was increased (>160 IU/ml) in 24 (57.1%) of the exposed workers. As shown in Table 1, all of the six asthmatic patients showed increased total IgE levels as well as blood eosinophilia, four had higher than 2,000 IU/ml. There may be a possibility that GD could directly activate mast cells, which result in the release of IL-4 and the induction of IgE synthesis. Further studies will be needed. However, significant associations were found between atopy or smoking, and specific IgE antibodies to GD in this study. These results suggested that atopy and smoking could be a predisposing factor in development of GD-induced asthma.

It was suggested that other inhalant allergens such as house dust mite, pollens, fungus and storage mite were included in grain dust and could act as allergens when exposed (11). Several investigators (12, 13) suggested that endotoxin included in the GD could induce airway inflammation. However, the present study revealed nine subjects with negative challenges contrasted with six asthmatic subjects with positive challenges. The skin test results to other common inhalant allergens suggest no association with skin reactivity to GD. Moreover, ELISA inhibition test results showed no significant inhibitions by the house dust mite or the storage mite allergens. These findings suggest that GD can act as an allergen itself. The possibility of contamination by other inhalant allergens or cross-reactivity with them seems to be extremely low.

In conclusion, we suggest that GD can induce an immunologic, IgE-mediated response in exposed workers, which is responsible for their asthmatic symptoms.

REFERENCES

1. DoPico GA, Reddan W, Flaherty D, Tsiatis A, Peters ME, Rao R, Rankin J. *Respiratory abnormalities among grain handlers: a clinical, physiologic, and immunologic study. Am Rev Respir Dis 1977; 115: 915-27.*
2. DoPico GA, Reddan W, Tsiatis A, Peters ME, Rankin J. *Epidemiologic study of clinical and physiologic parameters in grain handlers of northern United States. Am Rev Respir Dis 1984; 130: 759-65.*
3. Corey P, Hutcheon M, Broder I, Mintz S. *Grain dust elevator workers show work-related pulmonary function changes and dose-effect relationships with dust exposure. Br J Ind Med 1982; 39: 330-7.*
4. Yach D, Myers J, Brashaw D, Benatar SR. *A respiratory epidemiologic survey of grain mill workers in Cape Town, South Africa. Am Rev Respir Dis 1985; 131: 505-10.*
5. *British Medical Research Council questionnaire on respiratory symptoms, 1966.*
6. Chai H, Farr RS, Froelich LA, Mathison DA, Rosenthal RR, Shelter AL, Spector SL, Townley RG. *Standardization of bronchial inhalation challenge procedure. J Allergy Clin Immunol 1975; l56: 323-7.*
7. Park HS, Nahm DH. *New occupational allergens in a pharmaceutical industry: serratial peptidase and lysozyme chloride. Ann Allergy Asthma Immunol 1997; 78: 225-9.*
8. Akiyama K, Pruzansky JJ, Patterson R. *Hapten-modified basophilia model of human immediate hypersensitivity that can be elicited by IgG antibody. J Immunol 1984; 133: 3286-90.*
9. Park HS, Suh CH, Nahm DH, Cho SH, Kim YY. *Grain dust-induced occupational asthma: specific antibodies and basophil histamine releasability. Korean J Allergy 1996; 16: 396-406.*
10. Lams S, Wong R, Chan-Yeung M. *Nonspecific bronchial hyperreactivity in occupational asthma. J Allergy Clin Immunol 1979; 63: 28-34.*
11. O'Hollaren MT. *Baker's asthma and reactions secondary to soybean and grain dust. In: E.J. Bardana, A, Montanaro, M.T. O'Hollaren, editors. Occupational asthma, 1st ed. Philadelphia: Hanley and Belfus, Inc, 1992: 107-16.*
12. Jagielo PJ, Thorne PS, Kern JA, Quinn TJ, Schwarz DA. *Role of endotoxin in grain dust-induced inflammation in mice. Am J Physiol 1996; 270: 1052-9.*
13. Schwarz DA, Thorn PS, Jagielo PJ, White GE, Bleuer SA, Frees KL. *Endotoxin responsiveness and grain dust-induced inflammation in the lower respiratory tract. Am J Physiol 1994; 267: 609-17.*