J Korean Med Sci 1999; 14: 39-44
ISSN 1011-8934

Presence of Specific IgG Antibody to Grain Dust Does Not Go with Respiratory Symptoms

A high prevalence of work-related symptoms in relation to grain dust exposure has been reported in grain dust workers, but the role of the specific IgG antibody is unknown. To study the possible role of specific IgG (sIgG) and specific IgG_4 ($sIgG_4$) in the development of work-related symptoms, sIgG and $sIgG_4$ subclass antibodies against grain dust antigens were determined by ELISA in sera from 43 workers and 27 non-exposed controls. They were compared with results of specific IgE antibodies, exposure intensity and the presence of respiratory symptoms. SIgG and $sIgG_4$ antibodies were detectable in almost all sera of exposed workers, and the prevalence were significantly higher than those of controls ($p<0.05$). Higher $sIgG_4$ was noted in workers with specific IgE ($p<0.05$). The correlation between sIgG and exposure duration was significant ($p<0.05$). There was no association between the prevalence of sIgG and $sIgG_4$ and the presence of respiratory symptoms, or work stations. In conclusion, these results suggest that the existence of sIgG and $sIgG_4$ might represent a response to grain dust exposure and may unlikely play a role in the etiology of respiratory symptoms.

Key Words : *IgG; IgG_4; Grain dust; Inhalation exposure*

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Received : 19 June 1998
Accepted : 1 September 1998

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INTRODUCTION

Chronic inhalation of grain dust has been shown to cause acute and chronic airway injury characterized by bronchitis and airflow obstruction (1-4). Longitudinal studies have shown accelerated deterioration of pulmonary function in these grain workers (5). The severity appears to be related to the concentration of airborne grain dust in the work environment (4, 6). The presence of specific IgE antibodies in individuals who have clinically lower respiratory symptoms with exposure to grain dust, which has been demonstrated to have a prevalence of up to 40% in symptomatic workers in the same workplace, has been investigated (7).

The role of specific IgG (sIgG) remains to be determined in occupational asthma patients. Several studies (8-10) on laboratory animal allergy have reported the presence of sIgG antibodies in individuals exposed to animals, and some have suggested that the level of the sIgG antibody might reflect the level of exposure (8). Other studies dealing with reactive chemical-induced occupational asthma (11, 12) have reported a significant association between sIgG and the presence of work-related respiratory symptoms. However, in our previous study (7), three of six patients with grain dust induced asthma had high specific IgE antibody to grain dust. The failure to detect specific IgE antibody in the sera of three workers might indicate the involvement of another immunologic or non-immunologic mechanism in the induction of their asthmatic symptoms, since sIgG antibody has been suggested to have a role in the pathogenesis of asthma (13) in a situation where clinical sensitivity is suspected, but specific IgE antibodies cannot be detected.

In this study, in order to evaluate the significance of sIgG and $sIgG_4$ antibodies in grain dust-asthma, we studied the prevalence of grain dust-sIgG and $sIgG_4$ by ELISA in 42 grain dust-exposed workers and their relationships to respiratory dysfunction. The relationship between the existence of specific IgE and sIgG antibodies was also investigated.

MATERIALS AND METHODS

Subjects

All 42 subjects exposed to grain dust in our study 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 (Gilian, U.S.A.). Twelve employees were office workers and were classified as group I (low exposure group). Lower respiratory symptoms referred to cough, sputum, chest tightness, or shortness of breath. Symptomatic employees were those workers who had experienced lower respiratory symptoms during and after grain dust exposure. Atopy was defined as a positive reactor to more than one of the common inhalant allergens on skin prick test as described in our previous study (7).

Sera

Sera from 43 employees were collected and stored at -20℃, as well as sera from 27 individuals who had never been exposed to grain dust and who demonstrated negative skin tests to 50 common inhalant allergens including grain dust extracts as control subjects.

Preparation of extracts

Grain dust was obtained from the patient's workplace. It was extracted with phosphate-buffered saline [(PBS, pH 7.5), 1: 5 w/v] at 4℃ for 1 hr followed by centrifugation at 5,000 rpm. The supernatant was dialyzed (the cut-off molecular weight was 6,000 Da) against 4 liters of distilled water at 4℃ for 48 hr, and lyophilized at -70℃ for the preparation of antigens used in ELISA.

ELISA

A 96-well EIA flat-bottomed plate (Dynatec, MA, U.S.A.,) was filled with 10 μg/well grain dust antigens in a carbonate buffer (pH 9.6), and coated with the buffer only, which was determined before as the optimal concentration. After overnight incubation at 4℃, the plates were washed three times with 0.05 M Tween-phosphate-buffered saline (PBST). To each well was added 250 μL of 5% bovine serum albumin (BSA)-PBST, which was then incubated for 60 minutes at 37℃. All three assays were performed in triplicate.

Anti-grain dust IgG ELISA

Fifty microlitres of diluted patients' serum or negative control serum (1/200 in diluent buffer; PBST containing 3% BSA) were added to each well coated with grain dust. After incubation for 2 hr at 37℃, the wells were washed three times with PBST. One-hundred microlitres of horseradish peroxidase (HRP)-conjugated goat anti-human IgG (Sigma Co. U.S.A.) diluted into 1/2000 v/v with 3% BSA-PBST, were added to each well. The plates were then incubated at 4℃ for 2 hr. The wells were washed three times with PBST and then 50 μL of substrate solution were added, containing 0.01 M *o*-phenyl deamine-HCl in citrate phosphate buffer, pH 4.2, supplemented with 0.012% H_2O_2 before use. After incubation for 15 min at room temperature, 50 μL of 1 N H_2SO_4 was added to stop the reaction. The optical density of the solution at 490 nm was determined with a microtitre plate reader (MR 600, Dynamic Product, U.S.A.). The antibody titres were expressed as absorbances at 490 nm. The positive cut-off value was determined from the mean absorbance and three-fold standard deviation of 27 negative controls (mean + 3 S.D.=0.064). In all the final absorbance was obtained by subtraction of the absorbance from each uncoated well.

Anti-grain dust IgG_4 ELISA

Fifty microlitres of patient serum or negative control serum (undiluted) were added to each well coated with 10 μg/well of grain dust, and incubated for 2 hr at room temperature. After the wells were washed three times with PBST, 50 μL of biotin-conjugated mouse monoclonal anti-human IgG_4 (Sigma Chemical Co. U.S.A.) diluted to 1/1000 (w/v) with 5% BSA-PBST; they were incubated for 2 hr at 37℃. The wells were washed three times with PBST. Then, 50 μL of 1/1000 diluted streptavidin-HRP (Sigma Chemical Co. U.S.A.) were added and incubated for 30 min. The wells were washed three times and 50 μL of substrate solution (55 mg of 2,2'-azido-di-3 ethylbenzthiazoline sulphonic acid; Sigma Co.) in 100 mL of 70 mM citrate phosphate buffer were added to each well. After incubation for 5 min, 50 μL of 2 mM sodium azide was added to stop the reaction. The absorbance was measured at 410 nm with a microplate reader, and the antibody titres were expressed as absorbance values. The positive cut-off value was determined as 0.55 from the mean absorbance + 3 S.D. of 27 controls.

ELISA for specific IgE antibody to grain dust

The presence of specific IgE antibody to grain dust was determined by ELISA. The wells were incubated for 2 hr at room temperature with 50 μL of either the patients' sera (undiluted) or control sera. After washing three times with PBST, 100 μL of the 1:1000 v/v biotin-labelled goat anti-human IgE antibody (Sigma Co., U.S.A.) were added to the wells and incubated for 2 hours at room temperature. The wells were then washed three times with PBST and incubated with 1:1000 v/v streptavidin-peroxidase (Sigma Co., U.S.A.) for 30 minutes before another washing step which was followed by incubation with 50 μL ABTS (2.2'-azino-bis-3-ethyl-benzthiazoline sulfuric acid in a citrate phosphate buffer) for 10 min at room temperature. The reaction was stopped by adding 50 μL of 2 N sodium azide, and the absorbance was read at 410 nm by an automatic microplate

reader.

Statistical analysis

The x^2 and ANOVA tests were applied using the SPSS version 7.0 (Chicago) to evaluate the statistical differences among the data: p value of 0.05 or less was regarded as significant.

RESULTS

Immunologic studies

Fig. 1 and Fig. 2 illustrate sIgG and sIgG4 antibodies to grain dust in all employees and controls. Significant differences were noted between exposed workers and unexposed controls ($p<0.05$, respectively). Table 1 summarizes the prevalence of specific IgE, sIgG, and sIgG4 antibodies to grain dust. Of the 15 symptomatic workers, 6 (40%) had high specific IgE bindings, whereas 3 (11%) out of 27 asymptomatic workers were positive (p=0.02). None of the unexposed controls had the specific IgE antibody. While, there was no association between the presence of respiratory symptoms and the prevalence of sIgG and sIgG4 antibodies, in that 14 (93%) out of 15 symptomatic workers had sIgG, and 13 (87%) out of 15 symptomatic workers had sIgG4 antibodies to grain dust (p=0.95, p=0.80, respectively). Most asymptomatic workers had also sIgG (96%) or sIgG4 (93%) antibodies. Two individuals out of the 27 control patients who had never been exposed to grain dust had the sIgG antibody, and one had the sIgG4. There were no significant difference in the prevalence of sIgG and sIgG4 according to the presence of sIgE as shown in Table 2 (p=0.23, p=0.29 respectively).

Table 1. Association between respiratory symptoms and specific antibodies to grain dust

Respiratory symptoms	Prevalence		
	Specific IgE*	Specific IgG†	Specific IgG4‡
Positive (n=15)	6/15 (40)	14/15 (93)	13/15 (87)
Negative (n=27)	3/27 (11)	26/27 (96)	25/27 (93)
Unexposed controls(n=27)	0/27 (0)	2/27 (7)	1/27 (4)

*p=0.02, †p=0.95, ‡p=0.80

Table 2. Association between specific IgE and IgG or IgG4 antibodies to grain dust

Specific IgE antibody	Specific IgG antibody prevalence (%)	Specific IgG4 antibody prevalence (%)
Positive reactor	7/9 (78)*	7/9 (78)*
Negative reactor	32/33 (97)*	30/33 (91)*

*p=0.11, **p=0.28

Relationship between intensity and length of exposure, and serum specific antibodies to grain dust

In this study, 43 workers were classified into three groups according to exposure intensity at their workplace. The prevalences of sIgG and sIgG4 were not significantly different among the three groups, as shown in Table 3. The length of exposure varied from 1 to 13 years. There was a significant correlation between duration of grain dust expo-

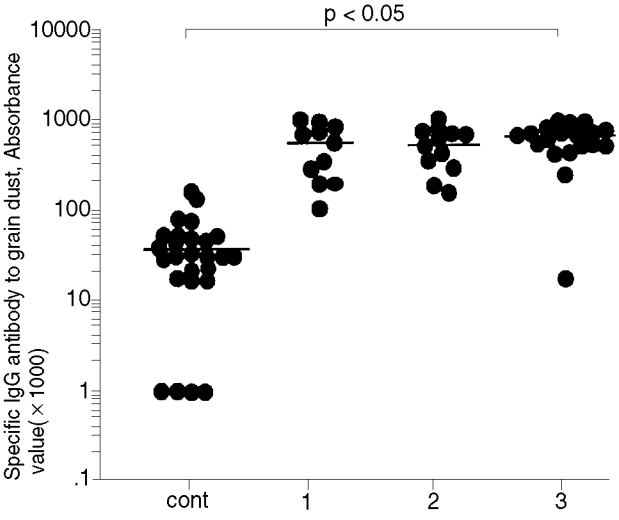

Fig. 1. Comparison of specific IgG to grain dust in three exposed groups and controls. Significantly higher levels were noted in exposed groups than in controls ($p<0.05$).

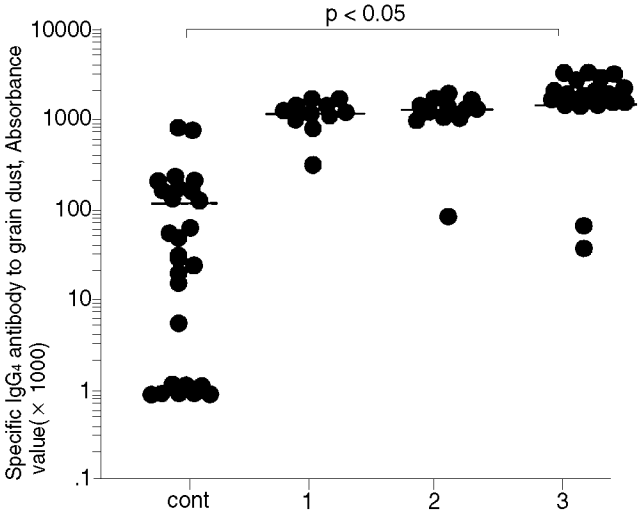

Fig. 2. Comparison of specific IgG4 to grain dust in three exposed groups and controls. Significantly higher levels were noted in exposed groups than in controls ($p<0.05$).

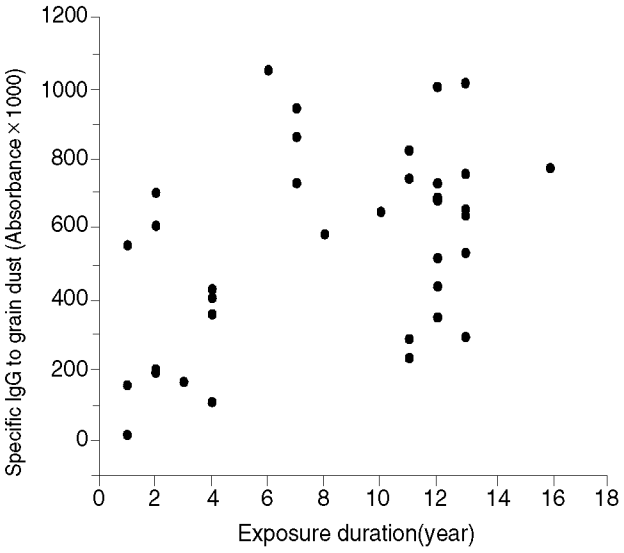

Fig. 3. Correlation between specific IgG to grain dust and exposure duration in the exposed group. A statistical significance was noted (r=0.41, p< 0.05).

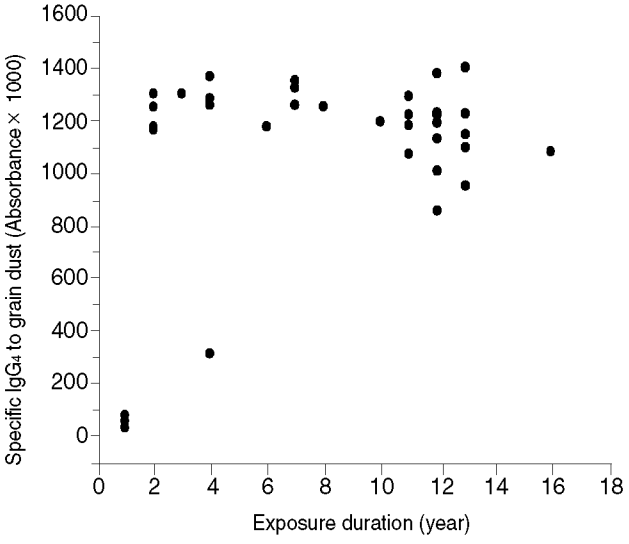

Fig. 4. Correlation between specific IgG4 to grain dust and exposure duration in the exposed group. A significant significance was noted (r=0.42, p<0.05).

sure, and sIgG and sIgG4 level [r=0.41 with sIgG (p<0.05 in Fig. 3), r= 0.42 with sIgG4 (p<0.05 in Fig. 4)].

DISCUSSION

The previous studies (7, 14) demonstrated serum specific IgE antibodies to grain dust by ELISA, and IgE-binding components within the grain dust extract by immunoblotting in the sera of asthmatic workers. This could prove that specific IgE is responsible for asthmatic symptoms in exposed workers. In this study, we demonstrated serum sIgG and sIgG4 antibodies to grain dust by ELISA, which proved suitable for IgG antibody detection. The sIgG response in other occupational asthma studies seems to be complex, and elicited a variable response to different antigens (15). Cartier et al. (11) noted that while the level of sIgG to isocyanate (hexamethylene diisocyanate and methylene diisocyanate) showed a satisfactory association with the results of specific inhalation challenges, the levels of specific IgE did not. Forster et al. (16) reported that sIgG to tetrachlorophthalic anhydride (TCPA) was detected in all asthmatic workers and some of the exposed asymptomatic workers. Moreover, the sIgG4 antibody increased in five out of seven occupational asthmatic workers due to TCPA, but was not detected in any of the asymptomatic individuals. Our previous study (12) on reactive dye asthma, revealed that the prevalence of sIgG and sIgG4 was significantly higher in symptomatic workers. They showed no association with exposure intensity within the same factory or duration of exposure. But the other report (17) comparing the prevalence of sIgG among reactive dye factory workers and those working in surrounding factories showed that the prevalence of sIgG remarkably decreased according to the distance from the reactive dye factory, suggesting that exposure intensity might be related to sIgG. However, several studies in other occupational settings have suggested that the presence of sIgG may represent a response to high dose exposure, not directly related with the development of respiratory symptoms. Quirce et al. (18), dealing with workers exposed to carmine dye, reported that all employees at that factory, regardless of their occupations or whether they had symptoms, had high levels of sIgG, probably as a consequence of the highly carmine-contaminated environment to which they were exposed. Similarly, the study (19) of sIgG in workers at the potato-processing industry demonstrated that sIgG was found in nearly all the workers, and a specific IgG4 subclass was found in about half of the workers, suggesting IgG4 as a

Table 3. Exposure intensity and specific antibodies to grain dust

Group	IgE antibody* prevalence (%)	IgG antibody† prevalence (%)	IgG4 antibody‡ prevalence (%)
CONT	0/27 (0)	1/27 (4)	2/27 (7)
I	3/11 (27)	10/11 (91)	10/11 (90)
II	0/12 (0)	12/12 (100)	11/12 (92)
III	6/19 (32)	18/19 (95)	17/19 (89)

*P<0.05, †P<0.05, ‡P<0.05
CONT, Control; I, Low exposure; II, Intermediate exposure; III, High exposure

predominant antibody. In this study, most workers regardless of respiratory symptoms had high sIgG and $sIgG_4$ antibody when they were exposed to grain dust and no association was found with the presence of respiratory symptoms. The correlation between length of exposure and sIgG was significant. These results suggest that the existence of sIgG and $sIgG_4$ to grain dust might represent a response to grain dust exposure and may not be related to respiratory symptoms.

Most of the IgE antibody responders were also $sIgG_4$ antibody responders, which suggest that specific IgE and $sIgG_4$ antibodies to grain dust might be a parallel immune response to inhaled antigens as other investigators have reported in grass and mite allergens (20-22). In the potato-processing worker's study (19), the possibility of $sIgG_4$ as a blocking antibody was reported, in which the level of $sIgG_4$ in symptomatic workers tended to be lower in non-symptomatic workers. In this study, there was no difference in absorbance level of sIgG and $sIgG_4$ between symptomatic and asymptomatic workers although ELISA is not enough to be applied in quantitation of absolute level of specific antibody.

The role of sIgG and $sIgG_4$ in grain dust asthma should be elucidated. Our previous study (14) revealed that sIgG to grain dust was detected in 100% of six occupational asthma patients proven by challenge tests. Indeed, 50% of them had grain dust specific IgE. The possibility of IgG_4 as a sensitizing antibody was suggested in a few occupational asthma settings (12, 23). There was one asthmatic worker who did not have sIgE, but had the serum $sIgG_4$ antibody. However, his basophils incubated with anti-IgG_4 antibodies did not release histamine, which suggested the possibility of the specific IgG_4 as a sensitizing antibody seemed to be minimal.

This study revealed no association between smoking and the relation of sIgG and $sIgG_4$ to grain dust. Comparable findings were found between smoking and the specific IgE antibody to grain dust in our previous study (7). Also, no association was found between atopy and sIgG and $sIgG_4$ antibodies, which was comparable to that of specific IgE in other studies (7, 24). This suggests that pre-existing atopy did not affect the development of sIgG and $sIgG_4$ antibodies to grain dust.

In conclusion, the existence of the serum specific IgG antibody to grain dust might represent exposure to grain dust. This response, however, is probably of no relevance to the occurrence of respiratory symptoms.

ACKNOWLEDGMENT

The authors are grateful to Ms Jinn Yoo and Mrs. Seo-Young Ji for their help in preparing this manuscript. This manuscript was funded by Ajou Post-Graduate School of Medicine (1996).

REFERENCES

1. Chan-Yeung M, Enarson DA, Kennedy SM. *The impact of grain dust on respiratory health. Am Rev Respir Dis 1992; 145: 476-87.*
2. DePico GA, Redden 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.*
3. DePico GA, Redden 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.*
4. Huy T, De Schipper K, Chan-Yeung M, Kennedy SM. *Grain dust and lung function. Dose-response relationships. Am Rev Respir Dis 1991; 144: 1314-21.*
5. Chan-Yeung M, Schulzer M, Maclean L, Dorken E, Tan F, Lam S, Enarson D, Grzybowski S. *A follow-up study of the grain elevator workers in the port of Vancouver. Arch Env Health 1980; 36: 75-81.*
6. Enarson DA, Vedal S, Chan-Yeung M. *Rapid decline in FEV1 in grain handlers. Relation to level of dust exposure. Am Rev Respir Dis 1985; 132: 814-7.*
7. Park HS, Nahm DH, Suh CH, Lee SH, Kim KS. *Occupational asthma and IgE sensitization to grain dust in exposed workers. J Korean Med Sci 1998; 13: 275-80.*
8. Platts-Mills TAE, Longbottom JL, Edwards J, Cockroft A, and Wilkins S. *Occupational asthma and rhinitis related to laboratory rats: serum IgG and IgE antibodies to the rat urinary allergen. J Allergy Clin Immunol 1987; 79: 505-15.*
9. Lee D, Edwards RG, Beeson MF, Dewdney JM. *Development and use of radiommunoassays for the detection of human IgG antibodies to laboratory animal urinary proteins. Int Arch Allergy Appl Immunol 1982; 69: 363-7.*
10. Schumacher MJ. *Characterization of allergens from urine and pelts of laboratory mice. Mol Immunol 1980; 17: 1087-95.*
11. Cartier A, Malo JL, Lagier F, Chezzo H, Harris K, Grammer L, Patterson R. *Specific antibodies against isocyanates (MDI, HDI and TDI): association with occupational asthma [abstract]. J Allergy Clin Immunol 1989; 83: 214.*
12. Park HS, Hong CS. *The significance of specific IgG and IgG_4 antibodies to a reactive dye in exposed workers. Clin Exp Allergy 1991; 21: 357-62.*
13. Akiyama K, Pruzansky JJ, Patterson R. *Hapten-modified basophil: a model of human immediate hypersensitivity that can be elicited by IgG antibody. J Immunol 1984; 133: 3286-90.*
14. 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.*
15. Price JA, Longbottom JL. *IgG antibodies in relation to exposure to laboratory-animal allergens. J Allergy Clin Immunol 1989; 84: 520-8.*

16. Forster MW, Venables KM, Nunn AJ, Newman-Taylor AJ, Topping MD. *Specific IgG and IgG4 antibody in workers exposed to tetrachlorophthalic anhydride [abstract]. Allergy 1988; 9: 294.*
17. Park HS, Kim JW, Hong CS. *The prevalence of specific IgE and IgG to reactive dye-human serum albumin conjugate in workers of a dye factory and neighboring factories. J Korean Med Sci 1991; 6: 63-8.*
18. Quirce S, Cuevas M, Olaguibel JM, Tabar AI. *Occupational asthma and immunologic responses induced by inhaled carmine among employees at a factory making natural dyes. J Allergy Clin Immunol 1994; 93: 44-52.*
19. Zock JPG, Doekes D, Heederik D, Van Zuylen M, Wielaard P. *Airborne dust antigen exposure and specific IgG response in the potato processing industry. Clin Exp Allergy 1996; 26: 542-8.*
20. Rowntree SR, Cogswell JJ, Michell EB, Platts-Mills TAE. *The development of IgE and IgG antibodies to food and inhalant allergens in children at the risk of allergic disease. Arch Dis Child 1985; 60: 727-35.*
21. Hill DJ, Hosking CS, Shelton MJ, Turner MW. *Growing out of asthma: clinical and immunological changes over five years. Lancet 1981; ii: 1359-62.*
22. Rawle FC, Burr ML, Platts-Mills TAE. *Long term falls in antibodies to dust mite and pollen allergens in patients with asthma or hay fever. Clin Allergy 1983; 13: 409-15.*
23. Park HS, Lee MK, Hong CS. *Specific antibodies to Black GR-HSA conjugates in occupational asthmatic patients to reactive dye, Black GR. J Korean Med Assoc 1989; 32: 1197-205.*
24. Blaski CA, Clapp WD, Thorne PS, Quinn TJ, Watt QJ, Frees KL, Yagla SJ, Schwartz DA. *The role of atopy in grain dust-induced airway disease. Am J Respir Crit Care Med 1996; 154: 334-40.*