

Identification of IgE-binding components in occupational asthma caused by corn dust

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Background: There have been some reports of corn dust-induced occupational asthma that suggest nonimmunologic mechanisms. In this paper, we present a case of occupational asthma and rhinitis caused by corn dust where bronchoconstriction was induced by an IgE-mediated reaction.

Methods and Results: The patient had positive responses to weed and grass pollens as well as corn dust extracts. The bronchoprovocation test elicited an early asthmatic response to corn dust extracts. Serum-specific IgE and IgG4 antibodies to corn dust extracts were detected by enzyme-linked immunosorbent assay (ELISA). In order to identify further the allergenic component of the extracts, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotting studies were performed. Ten IgE-binding components (9 to 140 kD) were detected within the corn dust extracts.

Conclusion: These results suggest that inhalation of corn dust can cause IgE-mediated bronchoconstriction in an exposed worker.

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INTRODUCTION

Studies have shown that chronic inhalation of grain dust has adverse effects on pulmonary health.¹ Inhalation of grain dust has been shown to cause acute and chronic airway injury characterized by bronchitis and airflow obstruction.² Immune mechanisms may be operative in some grain handlers who develop airflow obstruction^{3,4} but several lines of evidence indicate that the physiologic response to grain dust extract is mediated primarily by a non-immunologic inflammatory response in the lower respiratory tract.⁵⁻¹¹

A recent report¹² of cytokine patterns in bronchoalveolar lavage fluid cells from corn dust-induced occupational asthma patients suggests that a predominantly nonimmunologic mechanism, an acute inflammatory response to an inhaled toxin such as endotoxin, may be involved in the development of corn dust-induced bronchoconstriction. There have been few

reports regarding an IgE-mediated mechanism in occupational asthma caused by corn dust.

In this paper, we report a case of occupational rhinitis and asthma caused by corn dust, confirmed by bronchoprovocation test. Serum-specific IgE antibodies were detected and their IgE-binding components were identified.

CASE REPORT

The patient was a 24-year old non-smoking man, who was admitted to the Allergy Clinic of Ajou University Hospital in Suwon, Korea. Two years earlier, he began work that involved making stock feed. His job included mixing and pouring corn dust into a grain dust mixture. He experienced profuse rhinorrhea, sneezing, and chest tightness for a period of 6 months before admission to the hospital. He had never experienced such symptoms in the past. He complained that these symptoms were aggravated after handling corn dust. On admission, physical examination revealed no wheezing. Skin-prick tests elicited immediate positive responses to alder, oak, ragweed, timothy grass, blue grass, and

rye grass pollens as well as corn dust extracts. Radioallergosorbent test (RAST) revealed no significant responses as recorded in Table 1. Total IgE level by PRIST was 198 IU/mL. His chest and paranasal sinus radiographs were normal. Methacholine bronchial challenge test revealed a positive response at 25 mg/mL.

Preparation of Corn Dust Extracts

Corn dust, composed of ground-up kernels of corn, was obtained from his workplace. It was extracted in phosphate-buffered saline (PBS, pH 7.5), 1:10 wt/vol, 4°C for 24 hours, followed by centrifugation at 10,000 *g* at 4°C for one hour. The supernatant was dialyzed against distilled water and lyophilized at -70°C. For the skin-prick test, 1:5 wt/vol extracts were mixed with an equal amount of sterile glycerin.

Bronchoprovocation Test with Corn Dust Extracts

Airway responsiveness to methacholine was tested according to the method described previously.¹³ Bronchoprovocation tests were performed according to the procedure reported in

Table 1. Results of Allergy Skin Tests and RAST

Allergen	Skin reactivity mean diameter, mm		RAST
	Wheal	Erythema	
Mugwort	3	19	0
Timothy grass	1	5	1
Rye grass	1	5	ND*
<i>D. farinae</i>	1	5	0
<i>Tyrophagus</i> <i>putrescentiae</i>	0	0	0
Corn dust	2	10	
Rye dust	0	0	
Histamine	2.5	16	

ND: not done.

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the occupational asthma study.¹⁴ Normal saline was administered from a nebulizer 646 connected to a dosimeter (Devilbiss Co., USA). The pressure was 20 psi. The subject was asked to breathe the aerosol for two minutes with tidal breathing. This was followed by antigen extracts at concentrations ranging from 1:1000 wt/vol to 1:10 wt/vol. Pulmonary function tests including forced expiratory volume in one second (FEV₁) and FEF_{25-75%} were measured with a spirometer (Chest, Japan) before inhalation and ten minutes after each inhalation.

Then, the FEV₁ and FEF_{25-75%} were measured every ten minutes during the first hour. Pulmonary function tests were performed every hour for seven hours after the challenge.

ELISA for Specific IgE and IgG4 Antibodies to Corn Dust Extracts

The presence of specific IgE antibodies to corn dust extract was determined by ELISA. Microtiter plates (Dynatech, USA) were first coated with 100 μ L of corn dust extracts (5 μ g/well), and left at 4°C overnight. Each well was washed 3 times with 0.05% Tween-phosphate buffered saline (PBS-T), and the remaining binding sites were blocked by incubation with 250 μ L of 10% new-born calf serum for one hour at room temperature. The wells were then incubated for two hours at room temperature with 100 μ L of either the patient's serum (undiluted) or control sera from ten patients who had negative skin-prick test re-

sponses to common inhaled allergens as well as to corn dust extracts. After washing three times with PBS-T, 100 μ L of 1:1000 vol/vol biotin-labeled goat anti-human IgE antibodies (Sigma Co., USA) were added to the wells and incubated for two hours at room temperature. The wells were then washed three times with PBS-T and incubated with 1:1000 vol/vol streptavidin-peroxidase (Sigma Co., USA) for 30 minutes before another washing step followed by incubation with 100 μ L ABTS (2,2'-azinobis-3-ethyl-benzthiazoline sulfonic acid in a citrate phosphate buffer) for ten minutes at room temperature. The reaction was stopped by the addition of 50 μ L 2 M sodium azide and the absorbance was read at 410 nm by an automated microplate reader. All assays were performed in triplicate.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunoblotting

Corn dust extracts (10 mg/mL) were dissolved in a sample buffer (0.5 M tris-HCl, pH 6.8, 2.5 mL, glycerol 2.0 mL, 10% wt/vol sodium dodecyl sulfate 4.0 mL, 0.1% bromophenol blue 0.5 mL and distilled water 1.0 mL) and heated at 100°C for three minutes. Ten microliters of molecular weight markers (6 to 250 kDa) (Novex, San Diego, CA, USA) and antigen solutions were added to a Novex precast tris-glycine gradient gel (4% to 20% acrylamide) for the separation of antigens. Electrophoresis was performed with a Novex

Mini-cell for 90 minutes at 130 constant voltage. The gel was fixed and stained with Coomassie brilliant blue. For immunoblotting, the gel was soaked in a transfer buffer (12 mM tris, 96 mM glycine in 20% methanol) for ten minutes. Electroblothing was carried out for 90 minutes at 200 mA using a Novex western transfer apparatus. The blotted polyvinylidene difluoride membrane (Novex, USA) was then treated with PBS-T containing 5% bovine serum albumin for one hour to block non-specific protein binding. The patient's serum and control sera were diluted to 1:2 vol/vol with PBS-T. The membrane was then incubated with the patient's serum and control sera for 18 hours at room temperature. After being washed with PBS-T, the membrane was incubated with biotinylated anti-IgE antibodies (1:1000 wt/vol, Vector Laboratories, Inc., Burlingame, CA) for three hours at room temperature. The membrane was washed again with PBS-T and incubated for one hour with streptavidin-alkaline phosphatase (1:1000 wt/vol) (Sigma Chemical Co., St Louis, MO). As a substrate solution, 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (Sigma Co., USA) was applied to the membrane until positive bands appeared. After the reaction, the membrane was washed with distilled water and observed.

RESULTS

Bronchoprovocation Test with Corn Dust and Other Inhalant Allergen Extracts

As shown in Figure 1, there was no significant variation of FEV₁ on the control day when the subject was exposed to the diluent of 1:10 vol/vol extracts. An early asthmatic reaction was noted after the inhalation of undiluted extracts. Expiratory wheezing sounds were heard during the asthmatic response. In order to exclude bronchial sensitization to other inhalant allergens, bronchoprovocation test with mugwort and *D. farinae* extracts (Allergopharma, Germany) were performed which showed negative results.

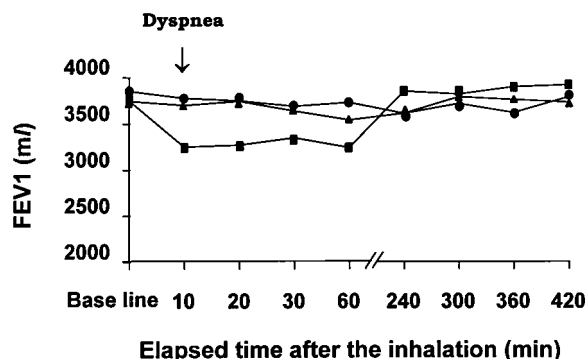


Figure 1. The results of bronchoprovocation test with corn dust extracts. ND: non-diluted. ●—● diluent, ▲—▲ 1:10 vol/vol corn dust, and ■—■ ND corn dust.

When bronchial challenge test with corn dust extract was performed in two house dust mite-sensitive asthmatic patients and ten symptomatic employees, all had negative responses.

Detection of Specific IgE and IgG4 Antibodies to Corn Dust Extracts

Figure 2a shows the specific IgE-binding to corn dust extracts in the patient's serum and control sera. In comparison to control results, highly specific IgE-binding to corn dust extracts was noted in the patient's serum. Figure 2b shows results of the ELISA inhibition test of corn dust, with the addition of mugwort, *Tyrophagus putrescentiae*, which is the most prevalent storage mite in this country, and *D. pteronyssinus* extracts as well as corn dust extracts. Significant inhibitions were noted with serial additions of the corn dust extracts. No inhibition was noted with other extracts. The absorbance value of specific IgG4 antibody with undiluted serum was 0.68.

Gel Electrophoresis

In order to determine the protein components of corn dust extracts, the extracts were analyzed by a 4% to 20% gradient SDS-PAGE. Figure 3a (left column) shows that the corn extracts resolved into more than ten bands ranging from 6 to 100 kD.

Immunoblot Analysis

Figure 3b shows the binding of specific IgE antibodies in the patient's (P) serum and control sera (C) on a blotted nitrocellulose membrane. With corn dust extracts, there were ten bands bound to IgE antibodies, with molecular weights ranging from 9 to 140 kD.

DISCUSSION

There have been several reports dealing with pathogenetic mechanisms of grain dust-induced occupational asthma. Immune mechanisms for grain dust asthma were suggested by few investigators.^{2,3} It was reported that measurement of specific IgE antibody to soybean could be a good marker for diagnosis of soybean asthma.⁴

Several investigators reported non-immunologic mechanisms in the inflammatory response of lower airways to grain dust. Atopic status and the presence of specific antibodies have not been consistently associated with either acute or chronic airway response to inhaled grain dust.^{5,6} There have been few studies suggesting an immunologic mechanism for corn dust-induced occupational asthma. In this study, the patient had occupational asthma and rhinitis due to grain dust, specifically corn dust, as confirmed by bronchoprovocation test.

The pathogenetic mechanism of bronchoconstriction may be an IgE-mediated reaction, since skin-prick test with corn dust extracts elicited an immediate positive response. Furthermore, IgE antibodies specific to corn dust extracts were detected by ELISA; the ELISA inhibition result suggests specificity of IgE-binding to this antigen. Neither crossreactivity nor contamination was suggested with pollens or mite antigens. Further, the immunoblotting study showed ten IgE-binding components within corn dust extracts. When bronchial challenge was performed on two house dust mite-sensitive asthmatic patients and ten symptomatic employees from in the same workplace, with airway hyperresponsiveness to methacholine, responses were negative, indicating that the bronchoconstrictive response in this patient was a specific one. These results suggest that corn dust can induce IgE-mediated bronchoconstriction in an exposed patient.

Some investigators have stressed that specific IgG4 antibody could be an exposure marker in workers exposed to laboratory animals.^{15,16} We noted the presence of specific IgG4 antibodies in sera of negative control patients as well as the study patient. Asymptomatic control patients who had negative

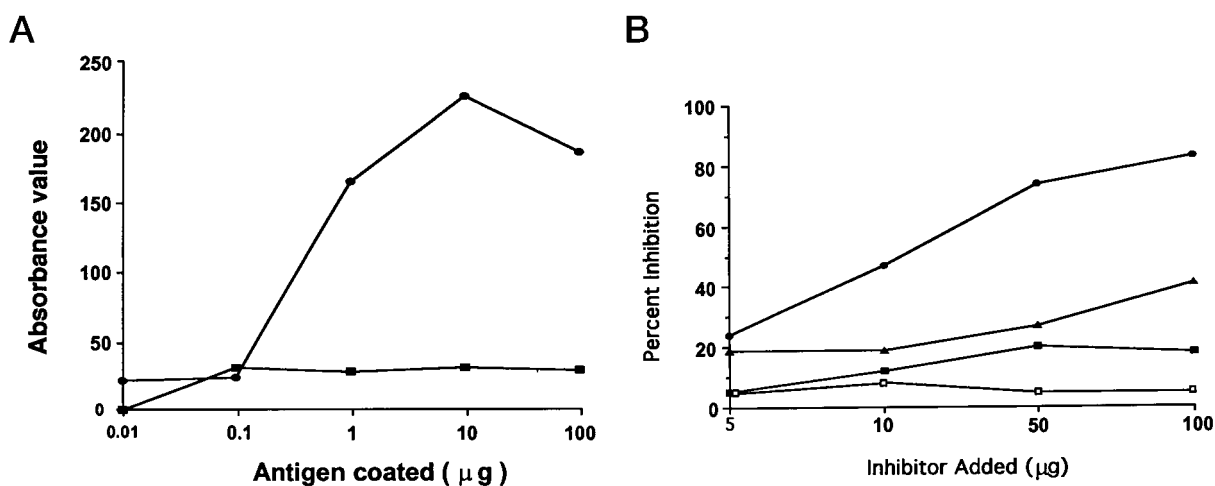


Figure 2a. Specific IgE-binding to corn dust extracts by ELISA in sera of patient and controls according to antigen concentrations. —●— corn dust and —■— control. Figure 2b. Corn dust-ELISA inhibition test with serial additions of house dust mite, storage mite, and mugwort pollen extracts as well as corn dust. ●—● corn dust, ▲—▲ mugwort pollen, ■—■ *D. pteronyssinus*, and □—□ *Tyrophagus putrescentiae*.

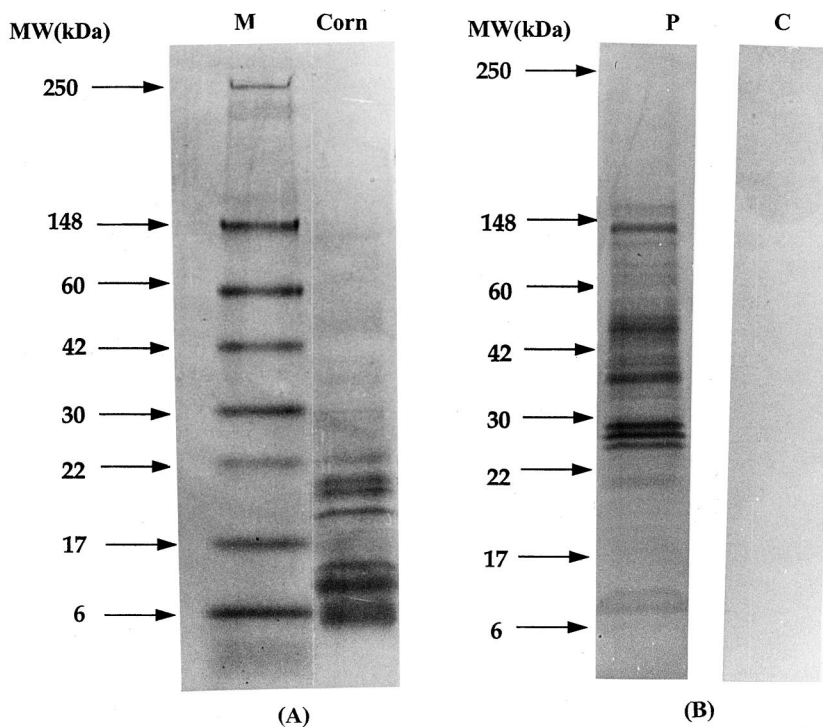


Figure 3. Four to twenty percent gradient SDS-PAGE (A) and immunoblotting (B) of corn dust extracts in sera of patient (P) and controls (C). M: marker protein, and C: corn dust.

skin-prick tests had high specific IgG4 antibodies without specific IgE antibodies.^{16a} These results suggest that frequent exposure to foods containing corn may induce production of specific IgG4 antibodies, which do not seem to be directly involved in the pathogenesis of respiratory symptoms.

Several authors argue for an important role of atopy in the development of IgE sensitization to occupational allergens with high molecular weight.^{15,16} Several studies have indicated a clear relationship between atopic status and grain dust-induced asthma.^{17,18} This corn antigen must be of macromolecular size since compounds with a molecular weight of less than 6000 to 8000 D were excluded by the dialysis membrane we used. The patient in this study was atopic, showing positive responses to pollen allergens on skin prick test but a negative response on mugwort-bronchoprovocation test, and he was a nonsmoker.

Diverse allergen sources may be included in the grain dust extracts.¹⁹ In

this study, the subject also showed positive responses to weed and grass pollens and minimal responses to storage mite and house dust mite on skin prick testing. ELISA inhibition test showed minimal inhibition by pollens, house dust mite, or storage mite antigens. These results suggested that the specific IgE-binding in this study was directed to corn dust, and there was little possibility of contamination by pollens or mites.

Several investigators suggest that neutrophils may have a major role in the development of asthmatic symptoms induced by grain dust. In vitro studies have demonstrated that grain dust can induce alveolar macrophages to release neutrophil chemotactic factor²⁰ and IL-1.

Bronchoalveolar lavage²¹ has demonstrated that the percentage of bronchial neutrophils significantly increases together with increased peripheral blood neutrophil and neutrophil chemotactic activity after grain

dust (sorgham dust) inhalation challenge.

Bronchoalveolar lavage of patients with corn dust-induced occupational asthma¹² showed a profound influx of neutrophils in the lower respiratory tract with marked increase in cytokine production (TNF α , IL-1 β , IL-1 receptor antagonist, IL-6, and IL-8). Clapp et al²² indicated that grain dust extracts had physiologic effects similar to those of endotoxin. In this study, the patient did not have peripheral neutrophilia and bronchoalveolar lavage disclosed 7% neutrophils, 3% eosinophils, 7% lymphocytes, and 83% macrophages. Further investigation will be needed to clarify the role of neutrophils in corn dust-induced occupational asthma.

In conclusion, this study demonstrates that corn dust can induce occupational asthma in an exposed employee working in the grain dust industry. Evidence presented indicates that an IgE-mediated immunologic mechanism may be involved.

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