# Occupational asthma and IgE sensitization to cellulase in a textile industry worker

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**Background:** Although there have been a few reports of occupational asthma due to cellulase in several occupational settings, this is the first case of cellulase-induced occupational asthma in an employee working in the textile industry. Its pathogenetic mechanism remains to be further clarified.

**Objective:** It is important to alert physicians to the possibility of occupational asthma caused by cellulase in workers of the textile industry.

**Methods and results:** The patient had atopy and strong positive responses to cellulase extract on skin prick tests. Bronchoprovocation test showed an early asthmatic response to cellulase extract. Serum specific IgE and specific $IgG_4$ antibodies to cellulase were detected by enzyme-linked immunosorbent assay (ELISA). In order to further characterize the allergenic component of the extract, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotting studies were performed. Eight IgE binding components ranging from 6 to 97.5 kD were detected within the cellulase extract.

**Conclusion:** These findings suggest that inhalation of cellulase can induce IgE-mediated bronchoconstrictions in employees working in the textile industry.

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## INTRODUCTION

Enzymes are high molecular weight (MW) proteins with catalytic activity and are suitable for a wide range of purposes. Inhalation of these enzyme dusts can lead to respiratory allergy.[1,2] Cellulase is a trivial name used in reference to a group of enzymes that catalyze the degradation of cellulose to glucose. The chemical name is $\beta$-1,4-glucan glucanohydrolase and it can be obtained from a variety of molds, mainly *Trichoderma viridae* and *Aspergillus niger* cultures. Cellulase is used as an important component of combined digestive aids, and other current applications are related to the removal or softening of unwanted cellulose from clothes.[2]

A few reports on allergic reactions caused by cellulase have been published,[2–5] although cellulase has had a long history of being used in digestive aids and in the foods industry.[2] The job categories of people who have worked with cellulase include laboratory assistants, process workers, pharmaceutical employees, and bakers.

In this paper, we report a case of occupational asthma caused by cellulase exposure in the textile industry. The bronchial sensitivity was confirmed by a specific bronchoprovocation test. Serum-specific IgE, $IgG_4$ antibodies and their IgE binding components were also identified.

## SUBJECTS AND METHODS

### *Case Summary*

The patient was a 23-year-old male worker, who was admitted to the Allergic Clinic of Ajou University Hospital, Suwon, Korea. Two years earlier, he had been employed at a textile company where his job responsibilities included dying clothes using reactive dyes and using cellulase to remove fuzz from the clothes on a daily basis. He had complained of cough and dyspnea over the previous 19 months. These symptoms were more aggravated at night after handling the cellulase powder during the day, but improved when he was on vacation. There were no skin lesions nor rhinitis symptoms. He had smoked a pack of cigarettes for 10 years, but stopped smoking 3 months ago because of dyspnea. On admission, physical examination showed clear breathing sound without wheezing. A skin prick test revealed positive immediate responses ($\geq 2+$ of A/H ratio) to *Dermatopgagoides farinae*, Fusarium spp., and cockroach (Bencard, Bredford, UK), suggesting atopic status, while negative responses to *Aspergillus fumigatus* and *Aspergillus niger* were noted. A skin prick test with 3 kinds of reactive dyes to which he had been exposed showed negative responses. A skin prick test with cellulase extract showed 3+ response. Not one of the 10 atopic or 10 non-atopic unexposed controls had a positive response to this extract. Total IgE level by radioimmunoassay (DPC, USA) was 681 IU/mL.

A chest radiography and EKG revealed no abnormal findings. He gave a written informed consent as regulated by Ajou University Hospital, Suwon, Korea.

### *Preparation of Cellulase Extract*

Cellulase powder was obtained from the patient's workplace. It was extracted with phosphate-buffered saline (PBS, PH 7.5, 1:50 wt/vol) at 4°C for 24 hours. The supernatant was dialyzed (cut-off molecular weight was 6 kD) against 1 L of distilled water at 4°C for 48 hours. For the skin prick test, the 1:5 wt/vol extracts were mixed with equal amount of sterile glycerine

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(final volume 1:10 wt/vol). The supernatant was used for the bronchoprovocation test at a concentration of 0.1, 1.0, 2.5 mg/mL.

*Inhalation Challenge test with Methacholine*

Airway responsiveness to methacholine was performed according to a method described previously with some modifications.[6] In this study, a methacholine challenge was performed one day before, and at 24 hours after the cellulase bronchoprovocation test.

*Bronchoprovocation Test with Cellulase Extract*

Bronchoprovocation tests were performed according to the procedure described previously.[7] Normal saline was administered from a nebulizer 646 connected to a dosimeter (Devilbiss Co, USA). The subject was asked to breathe the aerosol for 2 minutes with tidal breathing and, this was followed by antigen extracts at concentrations ranging from 0.1 to 2.5 mg/mL. The pulmonary function tests including forced expiratory volume in one second ($FEV_1$) and forced expiratory flow ($FEF_{25–75}$) were measured with a spirometer (Multispiro, USA) before and ten minutes after each inhalation. Then, $FEV_1$ and $FEF_{25–75}$ were measured every 10 minutes during the first hour. Pulmonary function tests were performed every hour for seven hours after the challenge.

*ELISA for Specific IgE and $IgG_4$ Antibodies to Cellulase Extract*

The presence of specific IgE and $IgG_4$ antibodies to cellulase was determined by ELISA. Microtitre plates (Costar, USA) were first coated with 50 $\mu$L of cellulase (2 $\mu$g/well) extract and incubated at 4°C overnight. Each well was washed three times with 0.05% Tween phosphate buffered saline (PBS-T) and the remaining binding sites were blocked by incubation with 350 $\mu$L of 1% bovine serum albumin-PBS-T for one hour at room temperature. The wells were then incubated for two hours at room temperature with 50 $\mu$L of patient serum (1:50 diluted) or control sera (1:50 diluted) from 4 patients who showed negative skin prick test response to common inhaled allergens as well as to cellulase. After washing three times with PBS-T, 50 $\mu$L of 1:500 vol/vol affinity purified biotinylated goat antihuman IgE antibody (Vector Lab, USA) or 1:2500 vol/vol biotin conjugate monoclonal mouse antihuman $IgG_4$ antibody (Sigma Chemical Co, USA) was added to the wells and incubated for 1 hour at room temperature. The wells were then washed 3 times with PBS-T and incubated with 1:1000 vol/vol streptoavidin-peroxidase (Sigma Chemical Co, USA) for 30 minutes followed by incubation with 50 $\mu$L *o*-phenylenediamine dihydrochloride for 10 minutes at room temperature. The reaction was stopped by addition of 50 $\mu$L 2.5N $H_2SO_4$ and the absorbance was read at 490 nm by an automated microplate reader. All assays were performed in duplicate.

*SDS-PAGE and Immunoblotting*

Cellulase was mixed with sample buffer (0.5M Tris-HC1, PH 6.8, 2.5 mL, glycerol 2.0 mL, 10% (wt/vol) SDS 4.0 mL, 0.1% bromophenol blue 0.5 mL and distilled water 1 mL), which was then adjusted to 1 mg/mL and heated in boiling water for 5 minutes. Ten microliters of standard marker (4 to 250 kDa, Novex, San Diego, USA) and antigen solutions were added to a Novex precast tris-glycine gel (12%) for the separation of antigens. Electrophoresis was performed with Novex Mini-cell for 90 minutes at 125 constant voltage. The get was fixed and stained with Coommasie brilliant blue. For immunoblotting, the get was soaked in a transfer buffer (25mM trisbase, 192 mM glycine in 10% methanol) for 10 minutes. Electroblotting was carried out for 90 minutes at 200 mA using a Novex Western transfer apparatus. The blotted polyvinylidine difluoride membrane (Novex, USA) was treated with 10% tris buffered saline-bovine serum albumin (TBS-BSA) solution for 1 hour to block non-specific binding. The patient's serum and control sera were diluted 1:1 vol/vol with 10% BSA-TBS. Each membrane was then incubated with patient and control sera overnight at room temperature. It was washed with 0.1% TBS-Tween (TBS-T) for 5 minutes. Affinity purified biotinylated goat antihuman IgE antibody (Vector Lab, USA) was diluted to 1:500 with TBS-T and used as a secondary antibody solution. The membrane was incubated with secondary antibody for 2 hours at room temperature and washed again.

Streptoavidin-alkaline phosphatase (Sigma Chemical Co, USA) was diluted to 1:1000 with TBS-T and added to the membrane for one hour. As a substrate solution, 5-bromo-4-chloro-3 indoly phosphate/ nitro blue tetrazolium (Sigma Chemical Co, USA) was applied to the membrane. After the reaction, the membrane was washed with distilled water and observed.

## RESULTS

*Bronchoprovocation Test with Cellulase Extract*

As shown in Figure 1, there was no significant variation of $FEV_1$ on the control day when the subjects were exposed to the diluent. Bronchoprovocation test was begun from 0.1 up to 2.5 mg/mL. There was no significant fall of $FEV_1$ after the inhalation of 0.1 and 1.0 mg/mL of antigen solution. After the inhalation of 2.5 mg/mL solution, early asthmatic reaction was noted as shown in Figure 1. To exclude the bronchial sensitization to reactive dye, which has been one of the common causative agents for occupational asthma in this country, reactive dye (Black B) bronchoprovocation test was performed on an individual day, which resulted in a negative response (data are not shown).

*Inhalation Challenge Test with Methacholine*

The initial methacholine challenge test revealed a positive response ($PC_{20}$ level: 6.8 mg/mL). When the second methacholine bronchial challenge test was done at 24 hours after the cellulase-bronchoprovocation test, there was an increase in airway hyperre-

sponsiveness to methacholine ($PC_{20}$ level: 0.94 mg/mL).

*Detection of Serum-Specific IgE and $IgG_4$ Antibodies to Cellulase Extract*

Figure 2 shows serum specific IgE and $IgG_4$ antibody bindings to cellulase extract in the patient serum and four unexposed control sera. In comparison to control sera, highly specific IgE and $IgG_4$ bindings to cellulase extract were noted in the patient's serum. Figure 3 reveals cellulase ELISA inhibition (Fig 3a: IgE ELISA inhibition test, Fig 3a: $IgG_4$ ELISA inhibition test) results with addition of cellulase extract. Significant inhibition was noted with serial addition of cellulase extract in IgE and $IgG_4$ ELISA inhibition tests, but minimal inhibition was noted with *Dermatophagoides farinae.*

*Gel Electrophoresis*

In order to determine the protein contents of cellulase extract, the extract was analyzed by a 12% SDS-PAGE. Figure 4a shows that cellulase extract resolved into eight bands ranging from 6 to 137.5 kD.

*Immunoblot Analysis*

Fibure 4b shows the binding of specific IgE antibodies in patient and control sera on the blotted membrane. With cellulase extract, there were eight bands bound to IgE antibody with molecular weights ranging from 6 to 97.5 kD, but no bindings with control sera.

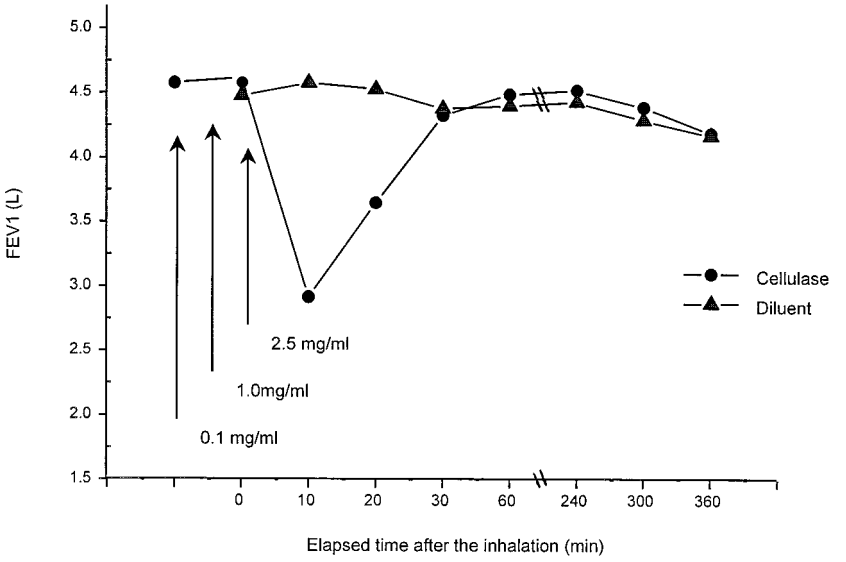



Figure 1. The result of bronchoprovocation test with cellulase extract (-●-) and diluent (-▲-).

*Clinical Course*

The patient was advised to discontinue exposure to cellulase and to take antiasthmatic medications. After 6 months′ avoidance, he had not experienced any asthmatic symptoms and his airway hyperresponsiveness to methacholine was improved ($PC_{20}$ level: 40 mg/mL).

## DISCUSSION

This study demonstrates that cellulase can induce occupational asthma in an exposed worker of the textile industry. This result was confirmed by specific bronchoprovocation test. Since the allergenic capacity of cellulase was first documented by Ransom and Schuster[3] in a pathologist working on a plant cloning experiment, several reports have pointed out its importance as an occupational inhalant allergen with job categories including laboratory assistants, process workers, pharmaceutical employees, and backers.

The pathogenic mechanism of occupational asthma caused by cellulase may be an IgE-mediated reaction,

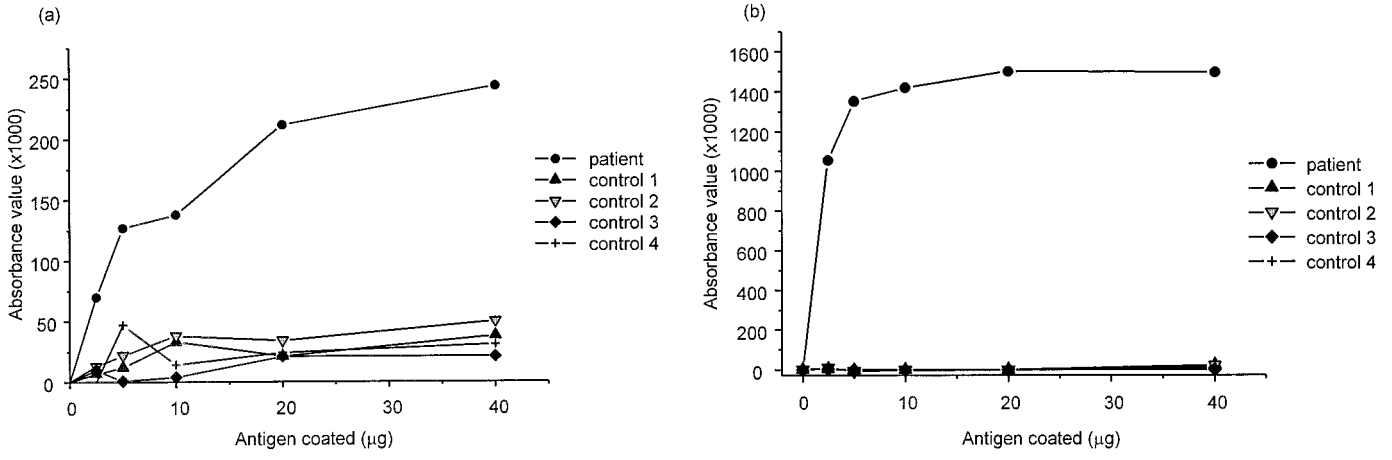



Figure 2. Specific IgE antibody (a) and $IgG_4$ antibodies (b) to cellulase extract in sera of patient and controls according to antigen concentration.

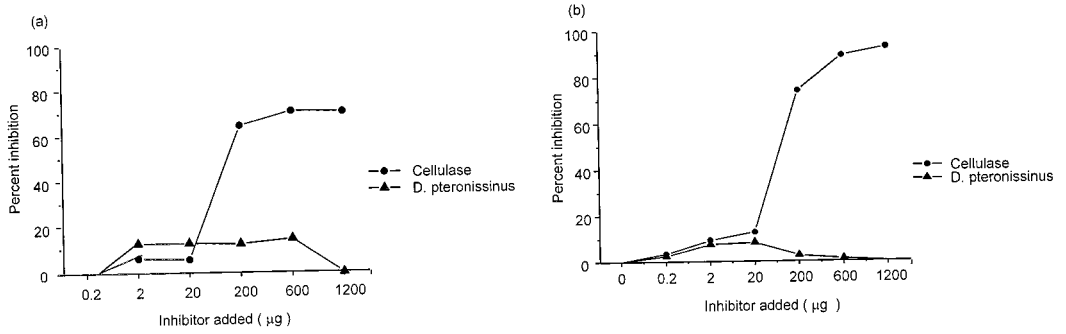



Figure 3. Percent inhibition of cellulase IgE-ELISA (a) and $IgG_4$-ELISA (b) with serial additions of cellulase and *D. pteronyssinus* antigens.

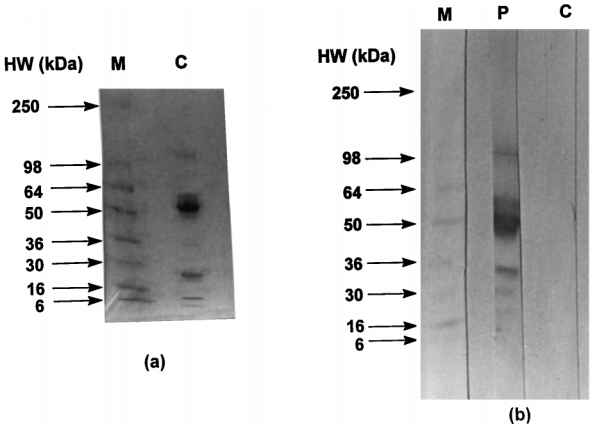



Figure 4. 12% SDS-PAGE (a) of cellulase extracts and immunoblotting (b) of cellulase extract in patient (P) and control sera (C). M = marker protein.

since skin prick and bronchoprovocation tests with cellulase extract on patients showed positive responses, while no responses were noted in unexposed controls. Furthermore, the presence of specific IgE in the patient's sera was demonstrated by ELISA. The ELISA inhibition test confirmed specificity of the assay for cellulase. The immunoblot study showed eight IgE binding components. In order to exclude non-specific effect of cellulase, the bronchoprovocation test with cellulase was performed in one house dust mite-sensitive asthmatic patient and he showed a negative response. These findings suggest that cellulase can induce IgE-mediated bronchoconstriction in an exposed patients. Reactive dye has been known as a common etiologic agent of occupational asthma in the Korean textile industry.[8] In the present study, we considered the possibility of sensitization to reactive dye (Black B) by doing a bronchoprovocation test with reactive dye that was derived from his work place, which resulted in a negative response.

There has been one study to observe an IgE-binding component with the cellulase extract. Quirce et al[5] reported several IgE binding components within the cellulase which was derived from Aspergillus spp. He demonstrated an allergen of MW 42 kD to 44 kD, a weak but clear band of approximately 85 kD, and a diffuse band of 62 to 75 kD, which probably consisted of two allergenic peptides. The cellulase used in this study was derived from *Trichoderma viride*, and *Fusarium moniliform*. There were at least 8 IgE binding components (97.5, 62 to 56, strong band 56–63, 35, 32.5, 24.5, 16.5 6) were noted within the cellulase extract. This finding may be caused by difference in source of cellulase used. Further investigation will be needed to extend the immunoblot assay in a large population.

The role of specific IgG and $IgG_4$ antibodies in occupational asthma has been studied.[9,10] The exact pathogenic role of these antibodies in the development of occupational asthma is still unknown. Some investigators suggested that these antibodies might be used as exposure markers but not have a pathogenic role in clinical situation.[11,12] In this study, compared with unexposed controls, the patient had a high serum-specific $IgG_4$ antibody that was significantly inhibited by ELISA inhibition test. Further studies will be needed to clarify the pathogenic role of specific $IgG_4$ antibodies in cellulase-induced occupational asthma.

There have been several reports that atopy plays an important role in the development of IgE sensitization to occupational allergens with high molecular weight.[13,14] This antigen must have been macromolecular in size because a compound with molecular weight of less than 6 kD was excluded by the dialysis membrane which we used. The patient had atopy and showed positive immediate responses to common inhalant allergens in our environment.

Cellulase could be obtained from various kinds of molds. Losada et al[2] demonstrated the cross reactivity between cellulase and an entire *Aspergillus niger* extract from which cellulase was derived by reverse enzyme immunoassay. This patient had a weak positive immediate response to Fusarium

spp. on skin prick test, but serum-specific IgE to Fusarium measured by RIA (DPC, USA) was negative. Although ELISA inhibition test was not performed with Fusarium spp. antigen, the possibility that his asthmatic reaction may have derived from crossreactivity with mold antigen seems to be very low.

In conclusion, this study demonstrated that cellulase induced occupational asthma in an exposed worker in the textile industry. Evidence is presented to indicate that IgE-mediated immunologic mechanisms may be involved in his asthmatic symptoms.

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