

Plasma Cluster Ions Reduce the IgE-Binding Capacity of House Dust Mite Allergens under a Simulated Indoor Environmental Condition

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Keywords

Air cleaning · Airborne allergens · Allergens · Environmental control · House dust mite · Plasma discharge · Positively and negatively charged cluster ions

Abstract

Background: A high level of house dust mite (HDM) allergens in a living environment is a risk factor for both sensitization to these allergens and asthmatic attacks. We previously showed that plasma cluster ions (PCIs) impaired the IgE-binding capacity of atomized crude allergens prepared from Japanese cedar pollen and fungus under experimental conditions. **Objective:** We evaluated the capacity of PCIs to impair the IgE-binding capacity of airborne HDM allergens under a simulated indoor environmental condition. **Methods:** For the determination of the effects of PCIs on HDM allergens under an experimental condition, HDM extract was atomized as aqueous mist into a cylindrical experimental apparatus filled with PCIs. For the evaluation of the effects of PCIs under a simulated natural indoor environmental condition, dried HDM allergens were floated as airborne particles in an acrylic cubic apparatus in the presence of PCIs. The IgE-

binding capacities of the PCI- and sham-treated HDM allergens were analyzed by an ELISA. **Results:** The IgE-binding capacity of the HDM allergens was significantly impaired after PCI treatment compared to that after sham treatment under both experimental and simulated environmental conditions. The ELISA results demonstrated that the IgE-binding capacities of HDM allergens after PCI treatment showed 68 and 74% reductions compared to those after sham treatment under the experimental and simulated environmental conditions, respectively. **Conclusions:** PCIs have the capacity to impair the IgE-binding capacity of airborne HDM allergens in a simulated environmental condition.

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Introduction

The number of individuals diagnosed with asthma has been steadily increasing worldwide, especially among children [1]. According to several asthma guidelines, the avoidance of indoor allergens, including house dust mites

H.O. and T.F. contributed equally to this work.

(HDMs) and pet dander, is effective for ameliorating the severity of asthma [2]. A double-blind placebo-controlled study showed that such allergen avoidance allowed a dose reduction of inhaled corticosteroid in asthmatic children [2, 3]. Another study showed that the concentration of HDM allergens in the bedroom during early life is significantly associated with sensitization to HDM allergens [4]. Importantly, early intervention by allergen-specific immunotherapy significantly improved the clinical response to HDM allergens in a nonrandomized open study [5]. These reports suggest that the avoidance of airborne allergens achieved by controlling the local environment and optimal treatment during early life may be important in preventing future allergen sensitization and the crisis of allergic asthma.

Der f 1 and Der f 2 are the most important HDM allergens for the diagnosis of HDM allergy because of their high sensitization and dominant IgE-binding capacity [6, 7]. Air pollutants such as nitrogen dioxide cooperate with Der p 1 to induce inflammatory cytokines from human nasal epithelial cells in nonatopic individuals [8]. Avoidance or inactivation of the major HDM allergens and elimination of the air pollutants from a living place during early life may be beneficial to avoid the future sensitization to HDM allergens.

In our previous studies, we employed a novel air-cleaning technology that emits both positively and negatively charged plasma cluster ions (PCIs) generated by plasma discharge, and we observed that the PCIs significantly reduced the IgE-binding capacity of atomized Japanese cedar pollen allergens and fungal allergens in an experimental cylindrical apparatus [9, 10].

PCIs can be generated by the discharge of plasma in a Plasma Cluster™ device. A plasma discharge produces OH radicals from atmospheric hydrogen oxide to generate positive and negative cluster ions [$\text{H}_3\text{O}^+(\text{H}_2\text{O})_m$ and $\text{O}_2^-(\text{H}_2\text{O})_n$ ($m, n \geq 0$), respectively], whereas the production of ozone and other ions is negligible [11]. Those OH radicals act on allergens to cleave their peptide bonds, and induce degradation and/or denaturation. The lifetime of the generated PCIs in air is approximately 3–5 s (unpubl. data). PCIs are not strong enough to destroy physical barriers or the lung epithelial cells of humans and animals, as they are not capable of damaging DNA or the cytoplasmic protein of bacteria and lung cells [12, 13]. Exposure to a 1,000-fold-higher level of PCIs (7,000,000 counts/ cm^3) than that normally observed in ordinary living conditions showed no toxicological or developmental effects on rats after 10 weeks of treatment [14]. These results indicate that PCIs are harmless to humans and animals

under ordinary conditions. Based on these results, in the present study we investigated whether the PCIs can reduce airborne HDM allergens in a simulated indoor environment.

Materials and Methods

Preparation of the HDM Extract

HDMs (*Dermatophagoides farinae*) were purchased from Osaka Kasei (Osaka, Japan), and the mite bodies were grown in a medium containing a 1:1 mixture of brewer's yeast (Asahi Food and Healthcare, Tokyo, Japan) and rodent diet (Oriental Yeast, Tokyo, Japan). The HDMs were separated from the culture medium as described [15]. Next, 2 g of HDM were placed in a sealed bag, heated in a microwave oven at 500 W for 2 min, and air dried overnight at 20–35°C. The dried mite bodies were crushed using a mortar and pestle. HDM extract was prepared by the addition of phosphate-buffered saline to the crushed dried mite bodies. The prepared HDM contained 288 µg of total protein and 0.8 µg of Der f 1 in 1 g of dry weight HDM.

Plasma from HDM-Allergic Patients

Plasma samples were obtained from 30 adult HDM-allergic patients with positive ImmunoCAP scores (3–6) to HDM and stored at –30°C until use. The experimental protocol was approved by the Institutional Review Board at Takanobashi Central Hospital. The experimental procedures were described in detail to all participants before obtaining their informed consent to participate in the study.

Experimental Chambers and Exposure to PCIs

For the experimental chamber analysis, we used a cylindrical experimental apparatus (14.5 cm in diameter, 52.5 cm high) as previously described [9]. The cylindrical apparatus was filled with PCIs generated from a PCI-generating device (a second-generation Plasma Cluster™ device, Sharp Corp., Osaka, Japan) for 15 min, followed by 8 mL of atomized 200 ng/mL HDM extract solution. The PCI-treated HDM solution was then collected in a petri dish at the bottom of the apparatus [9, 10]. A 1-m³ acrylic box equipped with a PCI-generating device (a second-generation Plasma Cluster™ device, Sharp Corp.) and a stirring fan were used for the evaluation of the effects of PCIs under a simulated indoor environmental condition (Fig. 2a). Enough PCIs were generated to maintain 3,000 PCIs/ cm^3 for 15 min in the cubic chamber, and then 0.5 g of dried HDM particles were scattered onto a cushion and spread into the chamber by beating the cushion. The airborne dry HDM particles were treated with 3,000 counts/ cm^3 of PCIs for an additional 15 min, and the particles were then collected on a membrane filter by pumping the air out of the chamber for 45 min. The collected HDM particles were suspended in 2 mL of phosphate-buffered saline to evaluate their IgE-binding capacity as a similar method reported in our previous studies [9, 10].

The lifetime of the generated PCIs in air is approximately 3–5 s (unpubl. data), so the residual PCIs had no effect on the following experiment without the continuous production of new PCIs. The safety test for the PCIs was entrusted to the Mitsubishi Chemical Safety Institute (Tokyo, Japan) and was carried out with reference to the OECD Principles of Good Laboratory Practice (C [97] 186/Final, 1997) [9].

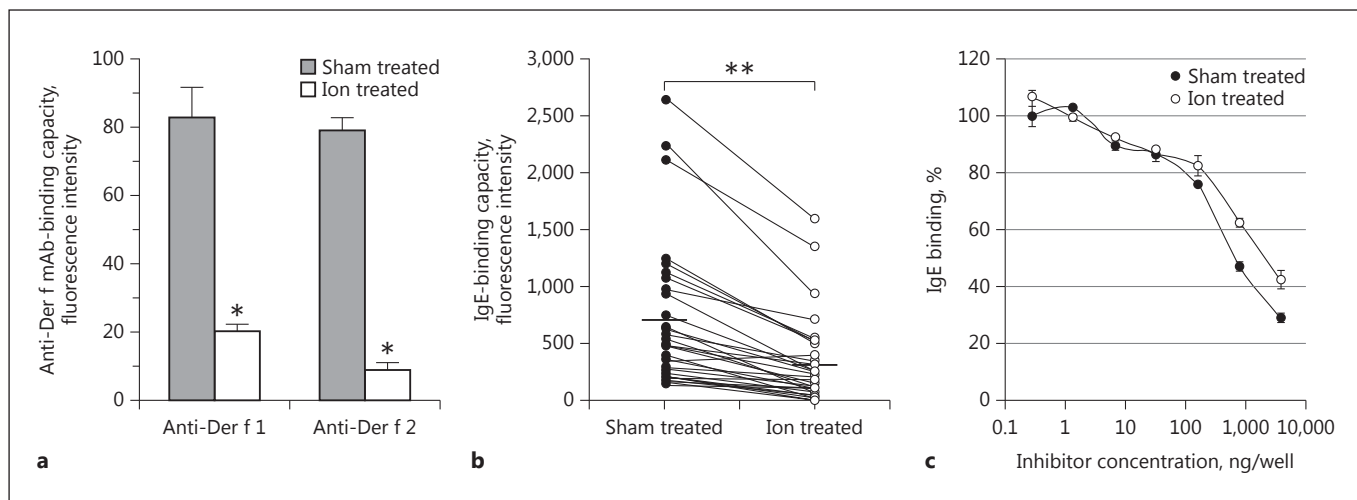


Fig. 1. Changes in the allergenicity of HDM allergens after PCI treatment. **a** The binding capacity of PCI-treated and sham-treated HDM extract with the mAb specific to Der f 1 or Der f 2 was analyzed by ELISA. * $p < 0.01$ by paired Student t test. **b** The IgE-binding capacity of PCI-treated and sham-treated HDM extracts in patients allergic to HDM ($n = 30$) was measured by ELISA. Each

circle represents an IgE titer for individual patients. ** $p < 0.001$ by Wilcoxon signed-rank test. **c** Impaired IgE-binding capacities of HDM extract after PCI treatment and sham treatment were confirmed by an ELISA inhibition assay using pooled plasma from HDM-allergic patients.

Enzyme-Linked Immunosorbent Assay and Inhibition Assay

We used an enzyme-linked immunosorbent assay (ELISA) to determine the IgE-binding capacity of the HDMs and the binding capacity to the corresponding monoclonal antibody of major HDM allergens, i.e., Der f 1 and Der f 2, in HDM extract after treatment with or without PCIs. The details of the ELISA are provided in the online supplementary materials (for all online suppl. material, see www.karger.com/doi/10.1159/000477724).

Statistical Analyses

All data for the antibody-binding assay are presented as the mean $\pm$ standard deviation. Data were analyzed using the paired Student t test or Wilcoxon signed-rank test. p values < 0.05 were considered significant.

Results

Atomized HDM extract was nebulized into a cylindrical chamber in the presence or absence of PCIs. After the provocation, we performed an ELISA to analyze the binding capacities between the major HDM allergens Der f 1 and Der f 2 and mAbs specific to the corresponding allergens. The ELISA results demonstrated that the binding capacities of HDM extract to the anti-Der f 1 and anti-Der f 2 mAbs were significantly abrogated after PCI treatment (Fig. 1a).

We then assessed the binding capacity of HDM extract with the patients' IgE after PCI treatment. We estimated

the IgE-binding capacities of the PCI-treated and sham-treated HDM extract by ELISA using sera from 30 HDM-allergic patients. The PCI-treated HDM extract demonstrated a significantly lower IgE-binding capacity compared to the sham-treated extract, and 87% of the patients (26/30) showed impaired IgE-binding capacity to the PCI-treated extract (Fig. 1b).

We further confirmed the impairment of IgE-binding capacity of the HDM allergens after PCI treatment by conducting an ELISA inhibition assay. We found that the concentration required for a 50% inhibition of binding between the pooled HDM-allergic patients' IgE and intact HDM extract was 2,005 or 635 ng for the PCI-treated and sham-treated HDM extracts, respectively (Fig. 1c). This result indicates a 68% reduction in the IgE-binding capacity of HDM allergens after the PCI treatment. These data clearly suggest that PCIs impaired the IgE-binding capacity of HDM allergens under the experimental condition.

We next examined the impairment of the IgE-binding capacity of HDM allergens after PCI treatment under a condition more similar to an indoor living environment by using a 1 m³ acrylic box (Fig. 2a). HDM allergens were floated in the box as dried particles, and we then exposed the airborne particles to PCIs. The PCI-treated HDM allergens showed a decreased IgE-binding capacity com-

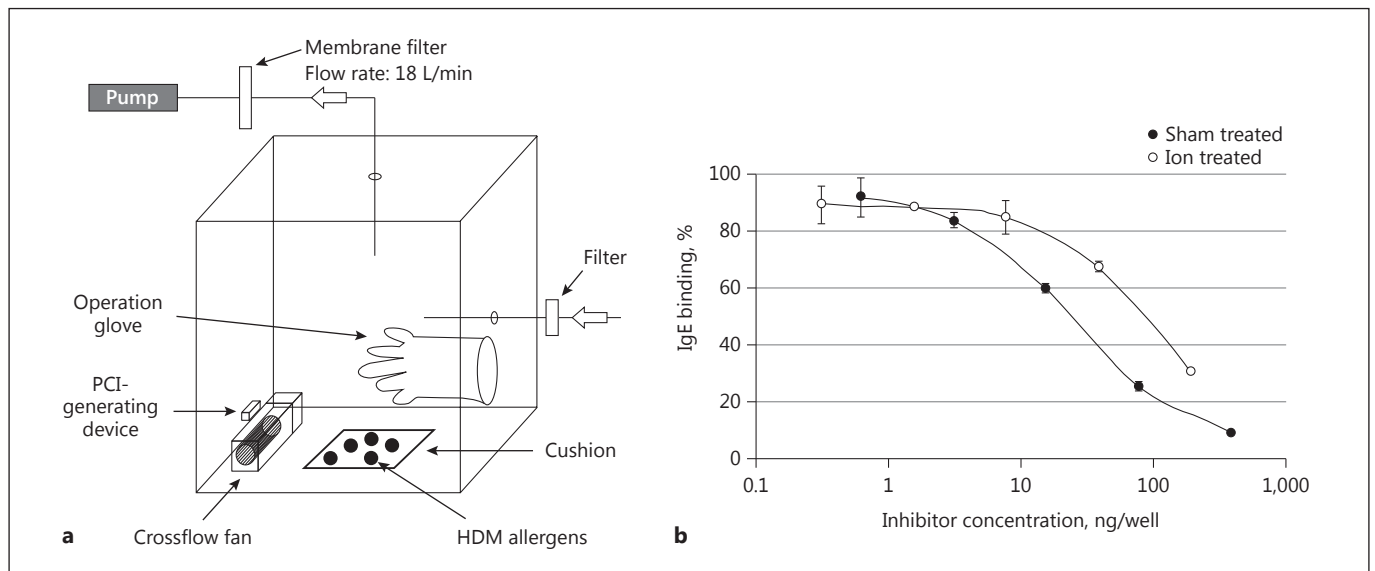


Fig. 2. PCIs impaired the IgE-binding capacity of airborne HDM allergens under the simulated indoor environmental conditions. **a** Apparatus for the simulated indoor environmental condition. The PCI-generating device ($2.15 \times 5.7 \times 3.1$ cm each) was placed in an acrylic box (1 m^3 each). PCIs were discharged from the PCI-generating device for 15 min. Next, 0.5 g of HDM particulate were

scattered onto a cushion and spread into the chamber by beating the cushion. The PCI-treated or sham-treated mite particles were collected on a filter attached to the top of the box with air flow. **b** The decreased IgE-binding capacity of the HDM allergens after PCI treatment was shown by an ELISA inhibition assay using PCI-treated and sham-treated HDM allergens as inhibitors.

pared to sham-treated HDM allergens in our ELISA inhibition analysis (Fig. 2b). The concentration required for the 50% reduction of IgE-binding with intact HDM extract was 87.5 or 24.5 ng for the PCI-treated and sham-treated HDM particles, respectively. The data showed that the IgE-binding capacity was impaired by 74% after PCI treatment. This result demonstrated that PCIs also decreased the IgE-binding capacity of dry HDM allergens under the simulated indoor environmental condition.

Discussion

We previously demonstrated that PCI treatment effectively inactivated atomized Japanese cedar pollen and fungal allergens [9, 10]. Those results prompted us to examine the effect of PCIs on HDM allergens, one of the most important airborne environmental allergens. Our present bench-level study using the experimental cylindrical chamber indicated that the PCIs decreased the IgE-binding capacity of major HDM allergens, Der f 1 and Der f 2, in an atomized HDM extract and impaired their IgE-binding capacity through protein degradation (Fig. 1 and data not shown). In addition, the exposure to PCIs

also significantly abrogated the IgE-binding capacity of dry HDM allergens under the simulated indoor environmental condition (Fig. 2). These data provide supportive evidence that PCIs inactivate airborne dry particle allergens similarly to atomized aqueous allergens.

We previously demonstrated that allergens from Japanese cedar pollen and fungus were degraded by PCI treatment in an experimental setting [9, 10]. In the present study we confirmed that the PCI treatment also degraded HDM allergens in a manner similar to that reflected by the previous studies' results, by conducting an SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) analysis (data not shown). We hypothesize that the mechanism of the PCIs to inactivate/denature airborne allergens is attributable to protein degradation by OH radicals generated by the Plasma Cluster device. However, further experiments are needed to clarify the accurate mechanisms for allergen inactivation/denaturation by PCIs.

For an effective inactivation of airborne allergens, it would be necessary to fill a living space with a high concentration of PCIs, e.g., $\geq 3,000 \text{ PCIs/cm}^3$, continuously. Although our present findings showed that PCIs can inactivate airborne allergens floating as dry particles in a

simulated 1-m³ acrylic box (Fig. 2a), it remains unknown how many PCIs and PCI-generating devices are needed to achieve a PCI concentration that is sufficient to inactivate airborne allergens in natural living environments. Therefore, clinical studies to investigate the effect of PCIs on natural airborne allergens in actual living environments are needed to evaluate the ability of PCI-generating devices to prevent HDM sensitization and asthmatic attacks in the future.

In conclusion, the results of this study indicate that air cleaning using PCIs has positive effects on the inactivation of natural HDM allergens under a simulated indoor environmental condition. It is necessary to further clarify the impact of environmental controls using PCIs on

HDM allergen sensitization and clinical symptoms, in clinical trials using PCI-generating devices.

Disclosure Statement

The second-generation Plasma Cluster™ device used in this study was provided by Sharp Corp., of which H.O. and K.N. are employees. All authors have no other conflicts of interest.

Funding Sources

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Online Supplementary Information

Enzyme-linked immunosorbent assay (ELISA) and ELISA inhibition assay

Briefly, 200 ng/ml house dust mite (HDM) solution in 100 mM bicarbonate buffer (pH 9.4) was added to the wells of a microtiter plate and incubated at room temperature for 2 hr. After the plate was washed three times with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBST), the wells were blocked with PBST containing 3% skim milk and 1% bovine serum albumin (BSA; Nacalai Tesque, Kyoto, Japan) at 4°C overnight. After the plate was washed with PBST three times, a mouse monoclonal antibody (mAb) specific to Der f 1 or a mAb specific to Der f 2 (200 ng/ml; Seikagaku, Tokyo, Japan) was added to the plate, which was then incubated at room temperature for 3 hr.

The plate was then incubated with 500 ng/ml biotinylated anti-mouse IgG1 mAb (BD Biosciences, San Diego, CA) for 1 hr, followed by incubation with alkaline phosphatase-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 hr. Finally, the plate was incubated with AttoPhos[®] Substrate (Promega, Fitchburg, WI), and the fluorescence intensity was measured using a Wallac 1420 ARVOsx Multilabel Counter (Perkin Elmer, Downers Grove, IL).

For the determination of the IgE-binding capacity of the PCI-treated and sham-treated HDM extracts, a 96-well microtiter plate was coated with 200 ng/ml HDM extract in 100 mM bicarbonate buffer (pH 9.4) at room temperature for 2 hr. After the plate was washed with PBST, the wells were blocked with PBST containing 3% skim milk and 1% BSA at 4°C overnight. After the plate was washed with PBST three times, 30-fold diluted plasma from mite-allergic patients was applied to the plate and incubated at room temperature for 4 hr.

For the quantitative evaluation of the effect of PCIs on the IgE-binding capacity of HDM allergens, ELISA inhibition analyses were performed with PCI-treated or sham-treated HDM extract. A microtiter plate was coated with 3.0 µg/ml HDM extract in 100 mM bicarbonate buffer (pH 9.4) and incubated at room temperature for 2 hr. After the plate was washed with PBST, the plate was blocked with PBST containing 3% skim milk and 1% BSA. Eighty-fold diluted pooled plasma from five mite-allergic patients was preincubated with serially diluted PCI-treated or sham-treated HDM extract at 4°C overnight.

The mixtures were then applied onto the HDM-coated microtiter plate and incubated at room temperature for 4 hr. Then plate was then incubated with 1,000-fold diluted biotinylated anti-human IgE mAb (Vector Laboratories, Burlingame, CA) at

room temperature for 2 hr, followed by incubation with alkaline phosphatase-conjugated streptavidin and AttoPhos[®] Substrate (Promega) for 1 hr. The fluorescence intensity was analyzed using a Wallac 1420 ARVOsx Multilabel Counter (Perkin Elmer).