

Decrease in the Allergenicity of Japanese Cedar Pollen Allergen by Treatment with Positive and Negative Cluster Ions

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Key Words

Allergenicity • Cluster ions • *Cryptomeria japonica* • Discharge plasma • Japanese cedar pollen

Abstract

Background: Japanese cedar pollinosis is a severe allergic disease in Japan. The most effective means of decreasing allergic inflammation reactions is still avoidance of the aeroallergen. Recently, a novel air purification system using positively and negatively charged cluster ions was developed to create comfortable living environments. We aimed to assess the ability of existing technology to lower allergenicity of Japanese cedar pollen. **Methods:** A Japanese cedar pollen extract was nebulized from the top of a cylindrical container with 2 or 4 ion-generating devices. The extract in a mist was passed through the space filled with or without plasma cluster ions for 90 s, and the ion-treated or nontreated extract was then collected in a Petri dish at the bottom of the container. **Results:** The ion-exposed extract was significantly diminished in its reactivities to anti-Cry j 1 or anti-Cry j 2 antiserum and to human allergic sera IgE on ELISA. SDS-PAGE analysis revealed that ion exposure induced protein degradation in the pollen extract. Similarly, the ion treatment impaired about 80% of the binding to pooled sera IgE from

patients allergic to Japanese cedar pollen on ELISA inhibition. Furthermore, intracutaneous and conjunctival reaction tests showed a remarkable diminution in the allergenicity of the ion-irradiated extract. **Conclusion:** Ion irradiation resulted in a remarkable decrease in in vitro and in vivo allergenicities of atomized Japanese cedar pollen extracts.

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Introduction

In proportion to increases in air pollution and allergen exposure, morbidity from allergic diseases such as rhinitis, dermatitis, and asthma is rising [1–4]. Especially Japanese cedar (*Cryptomeria japonica*) pollinosis has developed into a severe health and social problem, because the number of those allergic to Japanese cedar pollen (JCP) amounts to about 14% of Japan's total population [5].

The recognition that a pollinosis is provoked only by two major IgE-binding proteins, pectate lyase (Cry j 1) [6–8] and polymethylgalacturonase (Cry j 2) [9–12] has meant that the elucidation as regards other important allergens has been delayed. Recently, an isoflavone reductase-like protein [13] and chitinase [14] were characterized as additional allergens. We also found that 131 JCP

allergens were recognized in at least 1 of 40 sera IgE samples from patients with Japanese cedar pollinosis on two-dimensional immunoblotting, and that 4–86 protein spots (mean $\pm$ SD = 37 $\pm$ 22) among them reacted with the respective patient's IgE [15]. Although it is important to examine which allergen molecule plays a pivotal role in the elicitation of sensitization and allergic symptoms on the basis of its physiological properties, efforts to achieve this have become more laborious with the remarkable increase seen in sensitized allergens.

The environmental control of allergens has been recognized as an integral part of the overall management of allergen-sensitized patients [16]. Various trials designed to reduce exposure to indoor allergens appear indispensable to the maintenance of a high quality environment for patients. In most cases, allergen avoidance (e.g., from house dust mites, pets, cockroaches, and pollens) is accomplished by various cleaning measures within the house. Conflicting reports have been published about the effects of chemical expedients, carpet fresheners, acaricides, and protein-denaturing agents [17–21]. Physical means such as high-efficiency particulate arrest (HEPA) filter vacuum cleaners [22–24] and ionizers [25–28] seem to be more suitable for combating airborne allergens, but their clinical benefit is debatable. Accordingly, a combination of valid means directed at different sites seems a desirable approach, while more appropriate ways to effectively decrease the allergenic activities of airborne allergens in our living environment remain to be developed.

In this study, we utilized a novel air purification system emitting plasma cluster ions to significantly reduce the allergenicity of atomized JCP allergens floating in an atmosphere by passing them through a space filled with plasma cluster ions for about 90 s. We anticipate that this air purification system would have a high potential as an effective means to decrease exposure to airborne allergen for patients with a high risk for developing allergic symptoms.

Materials and Methods

JCP and Chemicals

JCP (*C. japonica*) was collected from Mikado Island, Hiroshima, Japan, in the year 2003 and stored at -30°C until use [13, 15]. All chemicals used were of analytical grade unless otherwise indicated.

Patients

C. japonica pollen-allergic sera were obtained from 40 subjects selected on the basis of clinical history, seasonal symptoms and

positive values against *C. japonica* pollen-specific IgE (RAST scores: 2–6), and stored at -30°C until use. All experiments were approved by the Institutional Review Board of Takanobashi Central Hospital and were described in detail to all participants before they provided informed consent and were included in the study.

Preparation of a JCP Extract

JCP (80 g) was suspended in 3 liters of 20 mM phosphate-buffered saline (pH 7.4) for 6 h at 4°C , and the suspension centrifuged at 9,300 g for 30 min at 4°C . Ammonium sulfate was added to the supernatant up to 80% saturation. The resultant precipitate was dialyzed against distilled water, and the dialysate was adjusted to 200 ng protein/ml and used as the JCP extract.

Structure of an Ion Generation Device and Determination of Ion and Ozone Densities

In this study we used an ion generation device attached to a domestic air cleaner (Sharp, Osaka, Japan) as described by Nishikawa and colleagues [29–31]. The device consisted of a ceramic dielectric plate, a high-voltage applied electrode, and a ground electrode. Plasma cluster ions were generated at atmospheric pressure by discharge plasma on the surface of the ceramic plate. The energy imparted to molecules in the air by discharge plasma was adjusted to about 5 eV. Ion densities were measured in terms of electrical conductivity of air on a double co-cylinder-shaped detector called a Gerdien condenser (83-1011B, Dan Kagaku, Tokyo, Japan). The density of generated ozone was measured either at a distance of 3 cm from the electrode for a duration of 90 min by an ozone monitor (EG-2001F, Ebara Jitsugyo, Tokyo, Japan) or in 600 ml of sampling volume for 6 min by an ozone detection tube (182U, Komyo Rikagaku Kogyo, Tokyo, Japan) immediately after the experiments.

Nontoxic Effects of Plasma Cluster Ions

The safety test 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)186Final, 1997]. In brief, rats (8-week-old, $n = 10$ each, male and female) were exposed to positive ions ($25,555/\text{cm}^3$) and negative ions ($28,010/\text{cm}^3$) for 6 h a day during a period of 28 days. No abnormalities or clinical signs occurred in any of the animals. There were no changes suggesting toxic effects of plasma cluster ion exposure on body weight, food consumption, hematology, blood chemistry, qualitative urinalysis, ophthalmoscopy, or organ weight. Furthermore, necropsy and histopathological examinations found no abnormalities in any animal.

Experimental Apparatus and Ion Exposure

An acrylic cylindrical plastic container (14.5×52.5 cm) was equipped with 2 or 4 ion-generating devices (fig. 1). When a high AC pulse voltage (width: 25 μs) was applied 120 times per second at 48, 80, or 140 AC input voltages between the electrodes, plasma cluster ions emerged from the surface of the ceramic dielectric plate. The lifetime of the generated plasma cluster ions in air was approximately 3–5 s. The density of generated ozone was measured at <0.005 , <0.005 , or 0.025 ppm at a distance of 3 cm from the device in proportion to the applied voltage. To exclude the additional effect of accumulated ozone in our closed experimental conditions, active carbon (Kishida Chemical Co., Osaka, Japan) was used. An ascending current of air was blown at a flow rate of

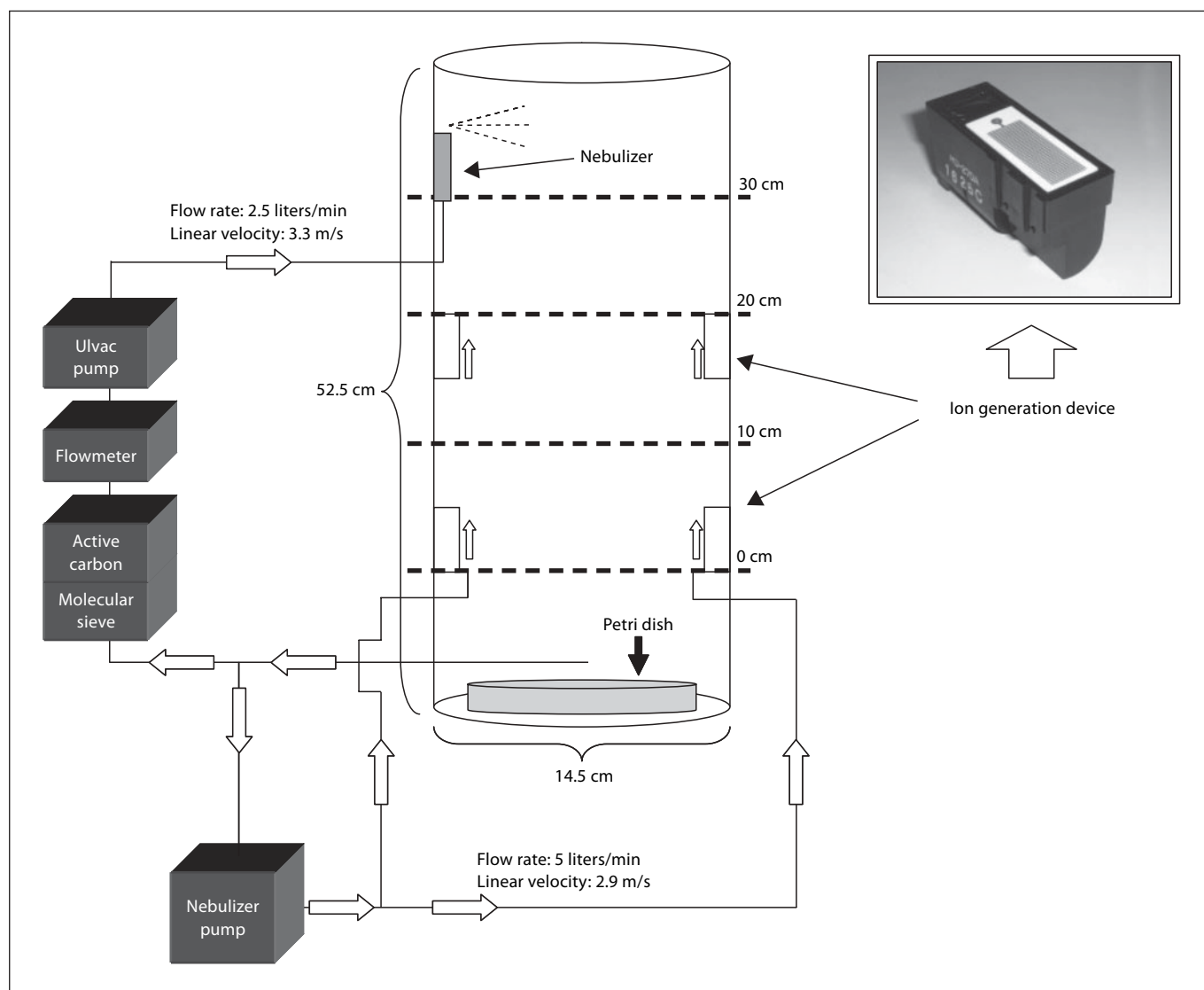


Fig. 1. Exposure system of plasma cluster ions to atomized JCP extract. Two or four ion generation devices ($2.15 \times 5.7 \times 3.1$ cm) were attached to a container ($\Phi 14.5 \times 52.5$ cm), which was filled for 15 min with plasma cluster ions. A JCP extract solution (200 ng/ml) was nebulized from the top of the container using a nebulizer pump and collected in a Petri dish at the bottom. An additional circulation pump was equipped to make an ascending current of air inside the chamber. Air flows are indicated by arrows.

5 liters/min from a nozzle installed on the surface of the ion-generating unit to disperse the plasma cluster ions and was then circulated from the outlet at the bottom of the chamber using a pump (from NE-c16 nebulizer, Omron, Kyoto, Japan). A molecular sieve 4A (Kishida Chemical Co.) and active carbon tubes were inserted in the circulating duct. The molecular sieve and charcoal were replaced every other run. After plasma cluster ions were pre-filled for 15 min in the chamber, 8 ml of JCP extract solution (200 ng/ml) was atomized for 1.5 h using a nebulizer pump (Ulvac pump DAP-15, Ulvac KIKO, Kanagawa, Japan). After 1.5 h irradiation, the densities of accumulated ozone measured at the bottom of the

chamber by an ozone detector 182U were 0.025 ppm in the chamber when input voltages of 4 ion-generating devices were set at 140 V, but were under the limit of detection in the other cases. A JCP extract in mist passed through a space full of or without plasma cluster ions for 90 s and was collected in a tray (about 1 ml) at the bottom of the container to serve as ion-treated or nontreated JCP extracts, respectively. After the ion-treated and nontreated experiments had been repeated 100 times, no significant difference in total protein contents (the method of Lowry et al. [32]) between the respective recovered and concentrated solutions was recognized. The effect of plasma cluster ions on allergen molecules was

evaluated in terms of changes in the intensities of antigenicity and allergenicity per unit of protein.

SDS-PAGE

Ion-treated and nontreated JCP extracts (50 µg protein each) were run on a 15% SDS gel under reduced conditions. Protein bands were visualized by silver staining.

Enzyme-Linked Immunosorbent Assay and Enzyme-Linked Immunosorbent Assay Inhibition

For determination of Cry j 1 and Cry j 2 contents in a JCP extract with or without ion treatment, 50 µl of the respective JCP extract solutions (100 ng/ml of 100 mM bicarbonate buffer, pH 9.4) was coated on a 96-well microtiter plate (F96 Cert. Maxisorp, Nunc-Immuno plate, Nalge Nunc International, Denmark) and incubated at room temperature for 2 h. After blocking the plate with 3% skim milk and 1% bovine serum albumin in PBST overnight, 1,000-fold diluted polyclonal antibodies against Cry j 1 or Cry j 2 (Seikagaku Kogyo, Tokyo, Japan) were added and incubated for 4 h. The plate was then incubated with horseradish peroxidase-conjugated anti-rabbit IgG (1,500-fold diluted, New England Biolabs, Beverly, Mass., USA) for 1 h. Next, the plate was stained with 50 µl of a substrate buffer containing 10 mg of ABTS [2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonate)] and 10 µl of 30% H₂O₂ in 10 mM citrate buffer at pH 4.2 and measured at 405 nm with a Wallac ARVO™SX plate reader (Perkin Elmer Japan, Yokohama, Japan).

The IgE-binding intensity of JCP extract exposed or not exposed to plasma cluster ions was evaluated by enzyme-linked immunosorbent assay (ELISA) [13]. In brief, 50 µl of a JCP extract solution (100 ng/ml of 100 mM bicarbonate buffer, pH 9.4) was coated on a 96-well microtiter plate and incubated at room temperature for 2 h. After blocking the plate overnight, 50 µl of 10-fold diluted patient sera was added and incubated for 4 h. Then, 50 µl of 1,000-fold diluted biotinylated anti-human IgE (ε-chain-specific, Vector Laboratories, Burlingame, Calif., USA) was added and incubated for 2.5 h. After washing the plate, alkaline phosphatase-conjugated streptavidin (50 µl, Jackson ImmunoResearch Laboratories, West Grove, Pa., USA) was added and incubated for 1.5 h. The washed plate was then stained with an Attophos™ substrate buffer (Roche, Mannheim, Germany) and measured at 485 nm emission and 530 nm excitation using a CytoFluor II microplate reader (PE Biosystems, Norwalk, Conn., USA).

ELISA inhibition was carried out with ion-treated or nontreated JCP extract solutions against pooled sera IgE (n = 5) from patients with Japanese cedar pollinosis [13]. In brief, 50 ng of an intact JCP extract in 50 µl of 100 mM NaHCO₃ at pH 9.4 was coated onto a 96-well plate for 2 h. Beforehand, 20-fold diluted pooled sera were reacted with 50 µl of ion-treated or nontreated JCP extract solutions at concentrations of 8 series of 5 times dilutions from a starting concentration of 11 µg/ml overnight at 4°C. The incubation mixture was added to the coated plate. Subsequent procedures were the same as described above.

Intracutaneous Test

Intracutaneous tests were carried out according to a previously reported method [33, 34]. Briefly, 20 µl of ion-treated or nontreated JCP extract solutions (500 ng/ml in 0.9% NaCl) were injected in patients with Japanese cedar pollinosis. Scores of –, ±,

+, ++ and +++ were assigned for erythema of <10, 10 to <20, 20 to <30, 30 to <40, and ≥40 mm in mean diameter, respectively, after 15 min.

Conjunctival Reaction Test

Conjunctival reaction tests were carried out in patients who received intracutaneous tests. In accordance with a previously described procedure [33, 35], one lower eyelid of each patient was challenged with 5 µl of a nontreated JCP extract in saline (5 µg/ml) as a control. The other eye of the patient was treated with the same amount of an ion-treated JCP extract. Conjunctiva was compared with the control eye to examine for congestion after 15 min. Scores of –, +, ++ and +++ were assigned for the degree of congestion observed as none, localized, intermediate, and strong, respectively.

Statistical Analysis

Data were analyzed by the difference between means. All experiments were repeated at least 3 times to confirm their reproducibility, and typical results are shown as figures.

Results

Density Distribution of Plasma Cluster Ions

Distribution of ion density was measured for 90 min under a circulating airstream (5 liters/min) in an acrylic cylindrical plastic chamber after two ion generation devices were run for 15 min (fig. 1). When the input voltage of the unit was set at 140 V, the densities of positive ions were detected at $10,070 \pm 64$, $40,010 \pm 70$, $80,040 \pm 120$ and $100,010 \pm 2,256$ counts/cm³ (n = 10) at a distance of 25, 20, 15 and 10 cm from the unit, while those of negative ions were at $10,020 \pm 87$, $49,960 \pm 102$, $79,980 \pm 117$ and $100,000 \pm 2,049$ counts/cm³, respectively. The ion densities were reduced with decreasing applied voltages (data not shown). To maintain a constant level of plasma cluster ion concentration in the experimental chamber, the number of ion generation devices was increased from 2 to 4 units.

Decrease in in vitro Antigenicities of JCP Extract Treated with Plasma Cluster Ions

First, we examined the change in rabbit IgG-reactive epitopes in Cry j 1 or Cry j 2 molecules induced by exposure of a JCP extract to plasma cluster ions generated from four ion-generating devices. Significant reduction in IgG-binding capacities of the major allergens, Cry j 1 and Cry j 2, was observed in ion-treated JCP extract (paired t test, p < 0.05), compared with nontreated JCP extract (fig. 2).

Next, we investigated whether changes in overall human IgE epitopes took place in JCP extract. The treatment of JCP extract with plasma cluster ions significant-

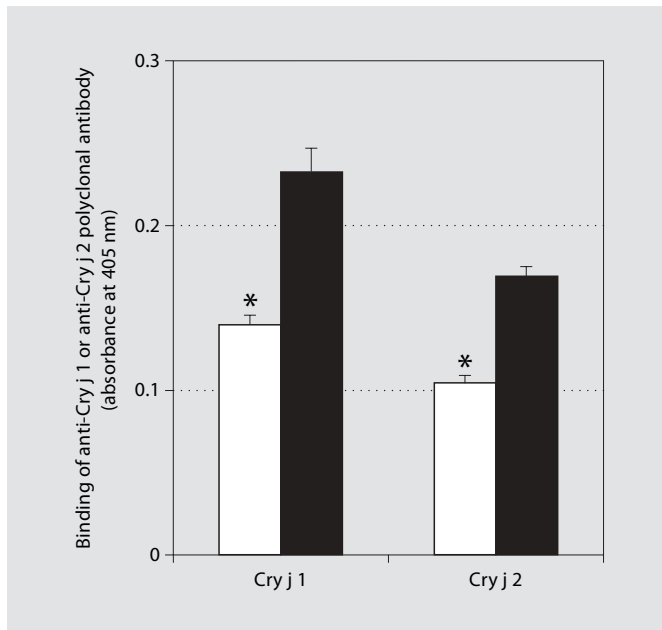


Fig. 2. Reduction in antigenicities of Cry j 1 and Cry j 2 in an ion-irradiated JCP extract. Changes in amounts of Cry j 1 and Cry j 2 recovered in Petri dishes after exposure of JCP allergen to plasma cluster ions (□) or without plasma cluster ions (■) were measured by ELISA using corresponding polyclonal antibodies. The experiment was repeated 3 times. * $p < 0.01$ (paired t test).

ly impaired the IgE binding activity in patients with Japanese cedar pollinosis in contrast with nontreated JCP extract (Wilcoxon signed-rank test, $p < 0.0001$, fig. 3). Furthermore, IgE binding was significantly diminished in 25 out of 40 samples (paired t test, $p < 0.05$, data not shown). SDS-PAGE analysis revealed that ion exposure induced protein degradation in the JCP extract (fig. 4).

To quantitatively evaluate the deterioration of IgE-binding ability, ELISA inhibition was carried out (fig. 5). Reaction of pooled sera IgE from patients with Japanese cedar pollinosis to nontreated JCP extract was completely inhibited by increasing concentrations of nontreated or ion-treated JCP extracts. The concentrations of the competitors, nontreated and ion-treated JCP extracts, required for 50% inhibition of IgE binding were estimated to be 8.4 and 40.5 ng, respectively. Thus, about 80% reduction of IgE binding was observed after exposure of the JCP extract to plasma cluster ions.

Decrease in in vivo Allergenicities of JCP Extract Treated with Plasma Cluster Ions

To compare in vivo allergenic activity between ion-treated JCP and nontreated JCP extracts, intracutaneous

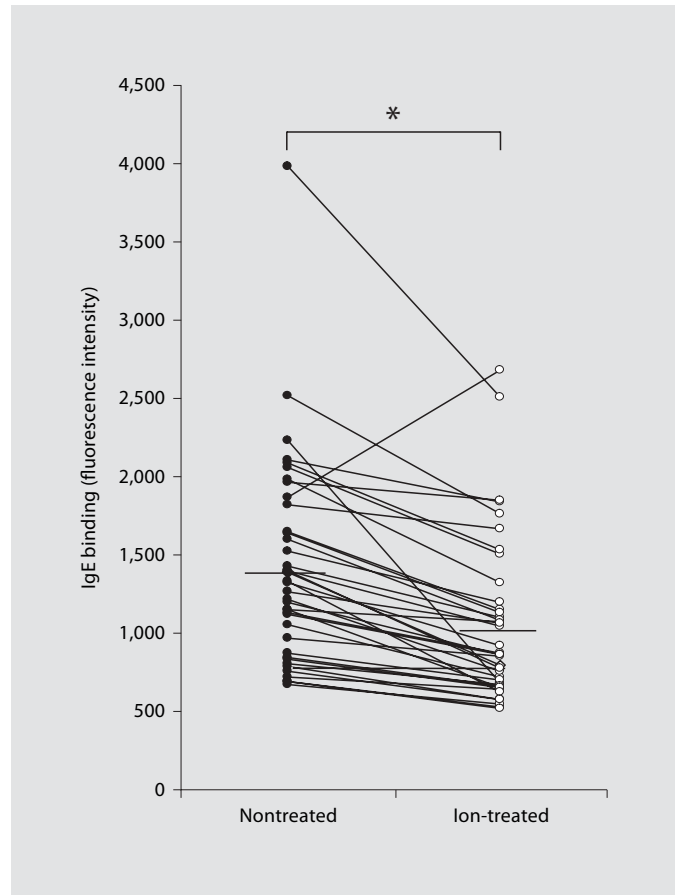


Fig. 3. Decrease in the ability of ion-treated allergens to bind IgE from patients allergic to JCP. IgE binding of ion-treated (○) and nontreated (●) allergens to sera IgE from patients ($n = 40$) sensitized to JCP was measured by ELISA. * $p < 0.0001$ (Wilcoxon signed-rank test).

reactivity was tested with 10 ng/20 μ l using patients allergic to JCP. The intracutaneous tests indicated that the allergenicity of the ion-treated JCP extracts decreased drastically from +++ to + in 8 subjects, from +++ to $\pm$ in 1 subject, and from ++ to + in 1 subject, compared to those of nontreated JCP extracts (table 1, fig. 6).

Table 1 also shows the result of the conjunctival reaction tests in the same patients. Ion-treated or nontreated JCP extract solutions (25 ng of protein/5 μ l) were dropped into either eye. The scores of conjunctival reaction after the ion treatment indicated that none of the patients tested experienced congestion.

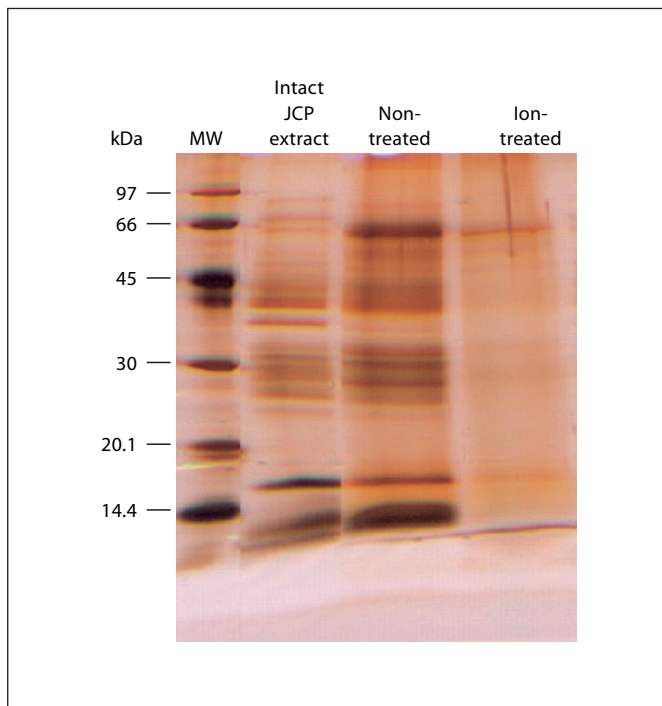


Fig. 4. Deterioration of protein bands in JCP extract by exposure to plasma cluster ions. Ion-treated and nontreated JCP extracts (50 μ g as protein) were run on a 15% SDS gel under reduced conditions. Respective protein bands were detected by silver staining.

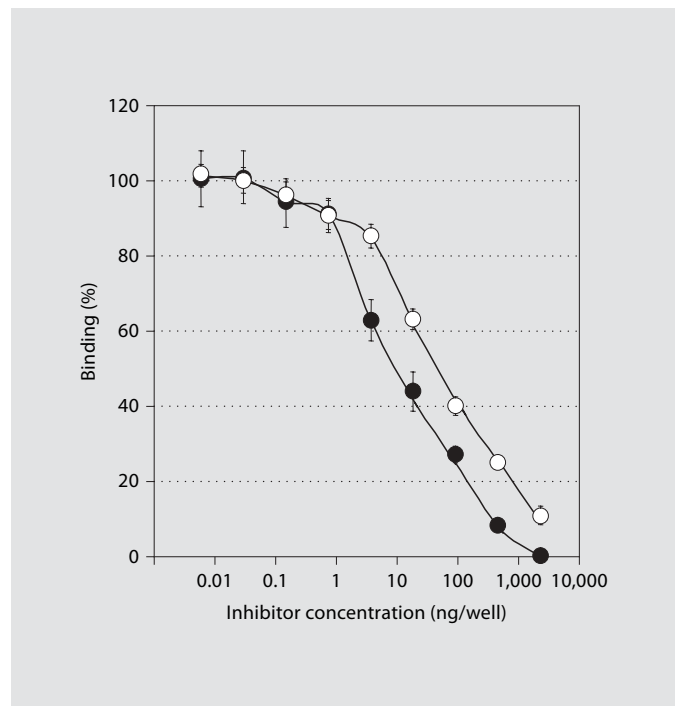


Fig. 5. Inhibition of JCP-allergic IgE binding to nontreated JCP extract with various concentrations of ion-treated or nontreated JCP extract. Pooled sera from patients allergic to JCP were preincubated with increasing concentrations of ion-treated (○) or nontreated (●) JCP extract. The incubation mixture was transferred to a well precoated with intact JCP extract. Then, IgE concentration bound to the well after the competition was determined by ELISA. Each point indicates the mean value from three independent wells and SD value is expressed by the error bar.

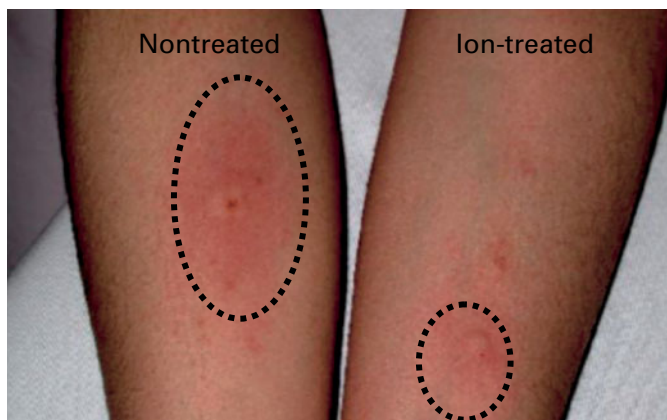


Fig. 6. Intracutaneous testing on forearms of JCP-allergic patients. Aliquots (10 ng/20 μ l) obtained after treatment with or without plasma cluster ions were injected intracutaneously. Patient A's left and right forearms show the results of intracutaneous tests using ion-treated (right) and nontreated (left) samples.

Discussion

Nojima and Nishikawa [31] reported that ion treatment for 15–60 min could abolish the proliferating ability of *Cladosporium* sp. spores and, from the disappearance of infectivity and hemagglutination reaction testing, might induce denaturation or decomposition of hemagglutinin on the surface of the virus [29]. Based on this evidence, we anticipated that ion treatment might be capable of lowering practical allergen levels to less than the threshold values eliciting allergic symptoms or sensitizations, because subtractive downmodulation of IgE-binding activity of ion-treated allergens would be dominant among three possibilities of their biochemical alterations, i.e. (1) inactivation or destruction of epitope structures, (2) formation of new epitopes, or (3) improved access of previously hidden epitopes [36–40].

Table 1. Skin test and conjunctival test of the ion-treated and nontreated JCP extracts

Patient	RAST (score)	Skin test ¹		Conjunctival test ²	
		ion- treated	non- treated	ion- treated	non- treated
A	5.78 (3)	+	+++	–	+
B	21.8 (4)	+	+++	–	+
C	64.0 (5)	+	+++	–	+
D	5.08 (3)	+	+++	–	–
E	50.4 (5)	+	+++	–	+
F	20.5 (4)	+	+++	–	+
G	80.7 (5)	+	+++	–	+
H	34.4 (4)	+	++	–	+
I	35.6 (4)	±	+++	–	+
J	34.1 (4)	+	+++	–	+

¹ Skin tests were conducted by injecting intracutaneously 20 µl of solution containing the ion-treated or nontreated JCP extracts (10 ng) dissolved in 0.9% NaCl. For erythema of 0–9, 10–19, 20–29, 30–39 or >40 mm in mean diameter, appearing after 15 min, a score of –, ±, +, ++ or +++ was assigned, respectively.

² One lower eyelid of each patient allergic to JCP was treated with a drop of a sample solution (25 ng/5 µl in 0.9% NaCl). After 15 min, the conjunctiva was compared with the control eye to examine for congestion. The symbols of –, +, ++, or +++ were assigned for the degree of congestion as follows: no, localized, intermediate, and strong, respectively.

In accord with a report that a sufficient amount of JCP capable of provoking allergic symptoms was present even in house dust before the pollen season [41], JCP was selected as our first choice of airborne allergens. Due to the inherent aerodynamic characteristics of JCP with a particle size of 30–37 µm, we were confronted with great difficulties in dispersing and floating JCP uniformly in a chamber. Indeed, JCP fell onto the bottom collecting dish as quickly as 2 s without wafting in the air under our experimental conditions. Similarly, a troublesome problem regarding a uniform suspension of powdered JCP extract also occurred. Accordingly, we could not help but use an atomized JCP extract as our next best choice in place of JCP. Further, the experiment using a nebulized JCP extract was considered to serve as a model reasonably similar to the actual phenomenon, because we could not recognize any remarkable differences in the reactions of plasma cluster ions to either wafted or atomized JCP extracts, although floated allergens would probably not be surrounded by liquid, since plasma cluster ions consist of positive and negative ions containing a cluster of water molecules.

The molecular structures of the plasma cluster ions generated were identified as $\text{H}_3\text{O}^+(\text{H}_2\text{O})_m$ and $\text{O}_2^-(\text{H}_2\text{O})_n$ ($m, n \geq 0$) as the major ions [31, 42], while ozone and the other ions were negligible [29–31]. The rate of decrease in in vitro antigenic activities (fig. 3, 4) and in in vivo allergic activities (table 1) was proportional to the densities of the plasma cluster ions caused by changing the input voltage. As a plausible explanation for the reduction in the antigenicity and allergenicity of JCP extract by treatment with plasma cluster ions, it is possible that the cleavage of various proteins by hydroxyl radicals, probably produced by collisions among cationic and anionic cluster ions, would occur because ion irradiation did not produce WST-1 formazan from a WST-1 solution, but generated formazan in the presence of H_2O_2 in a sealed dark reaction vessel and did not convert Amplex Red to resorufin in the presence of peroxidase (data not shown). Moreover, anionic cluster ions alone hardly reduced the antigenicity and allergenicity of JCP extract during short-term (90 s) contact.

Pollen and house dust mite and cockroach allergens only become airborne after vigorous disturbance and then settle rapidly [16]. Environmental modifications to limit exposure to these allergens should have significant potential to abate the prevalence of allergic diseases. Chemical treatments exhibited only temporarily beneficial effects against reservoirs of allergens but less against airborne allergens. Consequently, repeated application or additional approaches are required to control the bulk of allergens found in residences [18–21, 43]. Physical treatments seem more suitable against airborne allergens. HEPA filter cleaners have been shown to effectively remove airborne allergens; however, these devices were also shown to be ineffective in facilitating asthma control [22]. Airborne allergen avoidance using a HEPA filter has been similarly investigated with inconclusive and sometimes contradictory results [22–24].

Previous research on the use of negative ion generators that electrostatically precipitated airborne dust and allergens has shown little or no evidence of any clinical benefits from their use [25]. Warner et al. [26] reported that the use of ionizers could not be recommended in homes of asthmatic subjects to improve their symptoms, but that a significant abatement of airborne allergen concentrations may be useful as part of an allergen avoidance regimen. Ionizers and/or air filtration units did not reduce the levels of mite allergens in indoor environments, since the largest amounts of these allergens remained in settled dust [44]. The development of the most effective way to avoid becoming sensitized and provoking allergic symp-

toms would be to create the ideal circumstances of getting rid of all allergens from our living environments. Recently, Goodman and Hughes [27, 28, 45] developed a corona discharge ionizer and observed up to a 92% reduction in the concentration of settled Der p 1 in the presence of 2.40 ppm ozone for 3 weeks in an unoccupied but furnished office room. However, milder means to decrease allergen concentrations and so maintain the quality of daily life remain to be developed.

The significance of the results presented here is that the decrease in antigenicity and allergenicity of allergen can be achieved even by short-term (90 s) contact between nebulized JCP extract and plasma cluster ions. Recently, Takahashi et al. [46] reported that orbicles of 0.6- to 1.2- μ m size reside in a pollen-adhered form or a free form in quantities more than 8 times those of the pollen numbers scattering from the anthers. As it is conceivable from the particle size that an orbicle containing Cry j 1 would waft easily into a living environment, an orbicle might be more important in the induction of allergic

symptoms and more effective in its response to ion treatment than pollen.

The use of plasma cluster ions has much potential to inactivate the antigenicity and allergenicity of various airborne allergens without an elevation of ozone concentrations in domestic surroundings. From our preliminary results, we observed a similar decrease in specific IgE-binding activity to house dust mite allergens that became airborne after vigorous disturbance, from exposure to plasma cluster ions (3,000 counts/ml) for 30 min (data not shown). Further, the safety of successive exposures to the ions has been confirmed. Further research will be necessary to confirm the benefits, especially clinical ones, of plasma cluster ions under natural conditions in domestic environments.

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