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Reduction of Cry j 1 allergenicity by nano-sized electrostatic atomized water particles (NEAWPs) in human bronchial epithelium and dendritic cells

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Abstract
Japanese cedar pollen (*Cryptomeria japonica*) is a major cause of seasonal allergic rhinitis in Japan, and Cry j 1 has been identified as its principal allergen. Although current treatments provide symptomatic relief, they do not prevent sensitization, highlighting the need for novel preventive strategies. Nanosized electrostatically atomized water particles (NEAWPs) are nanometer-scale water droplets containing reactive oxygen species (ROS) that have demonstrated antimicrobial activity; however, their effects on airborne allergens remain unclear. Therefore, this study investigated whether NEAWPs could reduce the allergenicity of Cry j 1 in human bronchial epithelial cells (BEAS-2B) and monocytic antigen-presenting cells (THP-1). Briefly, Cry j 1 was exposed to NEAWPs for 24–48 h, and allergenicity was assessed by ELISA, calcium imaging, flow cytometry, and cytokine analyses. NEAWPs treatment markedly reduced Cry j 1 antigenicity in a time-dependent manner, achieving 98.6% reduction after 48 h. In BEAS-2B cells, untreated Cry j 1 induced significant Ca^{2+} influx and upregulated IL-8, TNF-α, and IL-6 expression, whereas NEAWP-treated Cry j 1 failed to elicit these responses. Similarly, in THP-1 cells, NEAWP-treated Cry j 1 decreased CD11c+CD86+ cells and inflammatory cytokine production. These results indicate that NEAWPs denature Cry j 1, preventing epithelial activation and antigen presentation, thereby attenuating downstream immune responses. This study provides the first evidence that NEAWPs can effectively reduce the allergenicity of Japanese cedar pollen, suggesting a potentially non-invasive, environmentally friendly approach for allergy prevention. Therefore, future studies are required to explore the molecular mechanisms, in vivo efficacy, and safety of NEAWPs exposure to establish its translational potential in managing pollinosis and other airborne allergies.

Keywords Nano-sized electrostatic atomized water particles (NEAWPs), Cedar pollen, Cry j 1, Bronchial epithelium, Dendritic cell

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1 Introduction

Allergic diseases represent a major public health challenge worldwide as they contribute to a substantial decline in quality of life and impose significant socioeconomic burdens [1–3]. The global prevalence of allergic rhinitis, asthma, and other allergic conditions has been steadily increasing over the past several decades, with pollinosis being one of the most common manifestations [4, 5]. In Japan, pollinosis has become a particularly serious health problem because of widespread exposure to Japanese cedar (*Cryptomeria japonica*) pollen, which is the leading cause of seasonal allergic rhinitis [6] (the Environmental Health Manual for Pollinosis 2022 published by the Ministry of the Environment, Japan). Epidemiological studies have shown that approximately 90% of patients with pollinosis in Japan are hypersensitive to cedar pollen allergens, highlighting the urgent need for improved strategies to prevent and manage this condition. Conventional treatments for pollinosis, including antihistamines and corticosteroids, provide symptomatic relief, but do not address the underlying causes of allergen sensitization. These medications often require continuous use during the pollen season, and their efficacy is limited to alleviating symptoms rather than preventing disease progression. In this regard, allergen-specific immunotherapy (AIT) shows promise as a causal treatment that modulates immune tolerance to allergens. However, AIT requires long-term administration, often spanning several years, and is associated with high costs and potential adverse effects. Additionally, patient compliance remains a major obstacle as the regimen can be burdensome and time-consuming. Physical protective measures such as face masks and eyeglasses are also widely used in Japan; however, their ability to completely block allergen exposure is insufficient. These limitations highlight the scarcity of accessible, effective, and practical approaches to treating pollinosis [7–10].

From the perspective of air-conditioning and indoor environmental control, airborne allergens constitute an important yet insufficiently addressed component of indoor air quality (IAQ). In recent years, advances in air-treatment technologies have expanded the scope of IAQ management beyond thermal comfort and particulate removal to include the inactivation of biological agents. One such innovation is the generation of nanosized electrostatically atomized water particles (NEAWPs). NEAWPs are formed by applying a high-voltage electric field to atmospheric water vapor, which results in electron-rich water clusters with diameters ranging from 5 to 20 nm [11]. These particles have been developed for indoor air-treatment applications and are currently implemented in air-conditioning and air-cleaning devices.

Owing to their nanoscale size and physicochemical properties, NEAWPs contain long-lived reactive oxygen species (ROS) that can disperse widely in indoor air. Previous studies have demonstrated that NEAWPs exhibit antimicrobial and antiviral activity, including inactivation of influenza virus, Escherichia coli, and SARS-CoV-2, primarily through oxidative damage to proteins and nucleic acids [12–15]. These findings support the use of NEAWPs as a non-invasive and environmentally friendly air-treatment technology for improving IAQ. However, despite their established efficacy against airborne pathogens, the effects of NEAWPs on non-infectious airborne agents such as allergens remain poorly understood.

Airborne allergens, similar to pathogens, interact with the respiratory epithelium and immune system and exert health effects through their biological activity rather than particle concentration alone. Therefore, from an IAQ engineering standpoint, evaluating allergenicity is a critical performance metric for air-treatment technologies. Whether NEAWPs can modify allergenic proteins and attenuate allergen-induced cellular activation has not yet been clarified. Addressing this knowledge gap is particularly important for Japanese cedar pollen, given its dominant contribution to allergic diseases in Japan.

Therefore, in this study, we investigated the potential of NEAWPs to reduce the allergenicity of Cry j 1, a major allergen derived from the Japanese cedar pollen. Using in vitro models of human bronchial epithelial cells (BEAS-2B) and monocytic antigen-presenting cells (THP-1), we examined the effects of NEAWPs-treated Cry j on cellular activation, calcium influx and cytokine production as biological indicators of allergen-inactivation performance relevant to indoor air-treatment applications.

2 Materials and methods

2.1 NEAWP generation

NEAWPs are water droplets containing reactive species such as hydroxyl and superoxide radicals. NEAWPs were generated using an electrostatic atomization device (Panasonic, Osaka, Japan). The device contains a Peltier element, which is electrically activated to cool the atomizing electrode, leading to the condensation of atmospheric water vapor on its surface. When a high voltage is applied between the atomizing and counter electrodes, the condensed water becomes electrically charged and migrates to the tip of the atomizing electrode, forming a Taylor cone. The electrostatic force at the cone tip exceeds the surface tension and gravitational forces, resulting in the ejection of fine droplets into the atmosphere. Owing to the electrostatic repulsion within the droplets, Plateau–Rayleigh instability occurs, fragmenting the droplets into nanometer-sized particles. As previously reported, the resulting particle sizes range from 5 to 20 nm, and the

NEAWPs exhibit strong oxidative properties because of the presence of long-lived ROS [12].

3 Treatment of Cry j 1 with NEAWPs

Japanese cedar pollen extract (Cry j 1; ITEA Inc., Tokyo, Japan) was dissolved in distilled water and freeze-dried in 40 mm Petri dishes. The dishes were placed in a 45 L box (380 × 344.5 × 387 mm) and exposed to NEAWPs from a distance of 5 cm above for 1, 24, or 48 h. Control samples were placed in the same chamber without NEAWP exposure (Fig. 1A). Treated and control Cry j 1 extracts were collected in distilled water. A portion of the extracts treated for 24 and 48 h was assessed for antigenicity using enzyme-linked immunosorbent assay (ELISA). Ninety-six–well plates were coated overnight at 4 °C with 50 µL/well of anti-Cry j 1 mouse IgE monoclonal antibody (10 µg/mL; BioDynamics Laboratory Inc., Tokyo, Japan) in PBS (pH 7.0). After washing, plates were blocked with PBST containing 1% bovine serum albumin (Sigma-Aldrich Co., LLC., Tokyo, Japan) for 1 h at 37 °C. Samples and standards (50 µL/well) were added and incubated for 1 h at 37 °C, followed by incubation with peroxidase-conjugated anti-Cry j 1 monoclonal antibody (1:1000 dilution; BioDynamics Laboratory Inc.) for 1 h at 37 °C. Plates were developed with ABTS (Sigma-Aldrich Co., LLC.) for 30 min at 37 °C, and absorbance was measured at 405 nm using a microplate reader (Multiskan FC; Thermo Fisher Scientific, Waltham, MA, USA).

4 Cell culture

The BEAS-2B cell line (European Collection of Cell Cultures, ECACC) was maintained in bronchial epithelial cell growth medium (BEGM™; Lonza Japan Ltd., Tokyo, Japan). The THP-1 monocyte cell line (ECACC) was cultured in RPMI 1640 medium (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) supplemented with 10% fetal calf serum (FCS; Sigma-Aldrich Co., LLC) and penicillin-streptomycin. Cells at ~70% confluency (1×10^5 cells/100 µL) were seeded into 3.5 mm glass-bottom dishes, 96-well plates, or 12-well plates and exposed to control or NEAWPs-treated Cry j 1 (0.5–0.75 µg/mL) for 24 h.

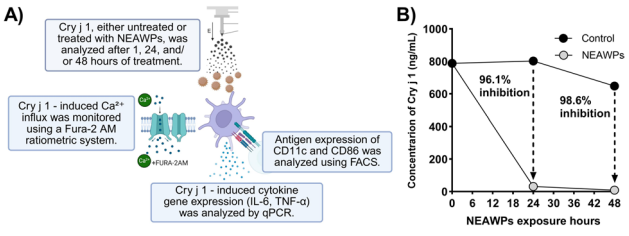


Fig. 1 Reduction in Cry j 1 concentration following NEAWP treatment. **A** Schematic illustration of Cry j 1 exposure to NEAWPs. **B** Japanese cedar pollen extracts were exposed to NEAWPs for 24–48 h, resulting in a time-dependent suppression of Cry j 1 antigenicity

5 Ca^{2+} imaging

Calcium imaging was performed in BEAS-2B cells exposed to NEAWPs-treated or control Cry j 1 (0.5 µg/mL) for 24 h, as described previously with modifications [16]. Cells were loaded with 2 µM Fura-2-acetoxymethyl ester (DOJINDO LABORATORIES., Kumamoto, Japan) for 30 min at 37 °C in darkness. After washing with HBSS, the glass-bottomed dishes were mounted on perfusion blocks and imaged under an inverted fluorescence microscope (Nikon Solutions Co., Ltd., Tokyo, Japan). Fluorescence was alternately excited at 340 and 380 nm, and emission was captured every 200 ms using a CMOS camera (ORCA-Fusion BT; Hamamatsu Photonics, Shizuoka, Japan). The cells were first perfused with Ca^{2+}-free Locke buffer (pH 7.4), followed by perfusion with Locke buffer containing $CaCl_2$ after 3 min. A temperature-controlled perfusion system was used to administer solutions.

6 Flow cytometry

THP-1 cells were exposed to NEAWPs-treated or the control Cry j 1 (0.75 µg/mL) for 24 h. Subsequently, surface expression of CD11c and CD86 was analyzed by staining with monoclonal antibodies (BioLegend, Tokyo, Japan) and DAPI. Samples were analyzed using a BD FACSAria III flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

6.1 Cytokine assays and gene expression analysis

Supernatant levels of IL-8 (BEAS-2B) and TNF-α (THP-1) were quantified using ELISA kits (DuoSet, R&D Systems, Minneapolis, MN, USA) and a microplate reader. Additionally, gene expression levels of TNF-α and IL-6 in BEAS-2B, and TNF-α, IL-6, and IL-1β in THP-1, were analyzed by qPCR. Total RNA was extracted using the NucleoSpin® RNA kit (TaKaRa Bio Inc., Shiga, Japan) and reverse transcription was performed using PrimeScript™ RT Master Mix (TaKaRa Bio Inc.). Subsequently, qPCR was performed using specific primers (TaKaRa Bio Inc.), PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific), and a CFX Duet Real-Time PCR System (Bio-Rad Laboratories, Tokyo, Japan). Relative expression levels were normalized to β-actin.

6.2 Statistical analysis

All data are presented as mean ± standard error of the mean (SEM). Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Šídák's multiple comparison test. Statistical significance was set at $p < 0.05$. significant. All data analyses were performed using GraphPad Prism version 10.5.0 (GraphPad Software, San Diego, CA, USA).

7 Results

7.1 Inhibition of Cry j 1 allergenicity by NEAWPs exposure

The direct effect of NEAWPs on the allergenicity of Japanese cedar pollen extracts was assessed using a Cry j 1–specific ELISA. NEAWPs treatment markedly reduced the Cry j 1 antigenicity in a time-dependent manner. After 24 h of exposure, the Cry j 1 concentration decreased by approximately 96.1% compared to the baseline, and after 48 h, the reduction reached 98.6% (Fig. 1B). These findings demonstrate that NEAWPs effectively inactivate Cry j 1 in vitro.

7.2 NEAWPs suppress Cry j 1-induced activation of BEAS-2B and THP-1 cells

We examined intracellular Ca^{2+} dynamics in BEAS-2B epithelial cells to evaluate whether Cry j 1 triggers a Ca^{2+} influx, similar to that by other airborne allergens such as house dust mite. The control Cry j 1 (untreated) significantly increased Ca^{2+} influx in both cell types compared to the untreated medium controls. However, NEAWP-pretreated Cry j 1 failed to induce Ca^{2+} influx, with levels comparable to baseline (Fig. 2A-C). In addition, Cry j 1 exposure upregulated ratio of $CD11c^{+}CD86^{+}$ cells in THP-1 cells, indicating an enhanced antigen-presenting capacity. NEAWPs treatment significantly attenuated Cry j 1–induced increase of $CD11c^{+}CD86^{+}$ cells compared with the control Cry j 1 group (Fig. 2D).

7.3 NEAWPs attenuate Cry j 1-induced inflammatory cytokine responses

Next, we investigated whether NEAWP treatment altered Cry j 1 -induced cytokine responses. In BEAS-2B cells, Cry j 1 significantly increased IL-8 production compared to that in the untreated controls, however, NEAWP-treated Cry j 1 failed to elicit this response (Fig. 3A). Consistently, mRNA expression of TNF-α and IL-6 was significantly suppressed following NEAWP treatment (Fig. 3B, C). Similarly, in THP-1 cells, Cry j 1 exposure enhanced TNF-α secretion and upregulated TNF-α, IL-6, and IL-1β gene expression. These effects were significantly attenuated when Cry j 1 cells were pretreated with NEAWPs (Fig. 3D–G). Taken together, these results indicated that NEAWPs effectively reduced the allergenicity of Cry j 1 by inhibiting its ability to induce calcium influx, CD11c and CD86 upregulation, and inflammatory cytokine responses in airway epithelial and antigen-presenting cells.

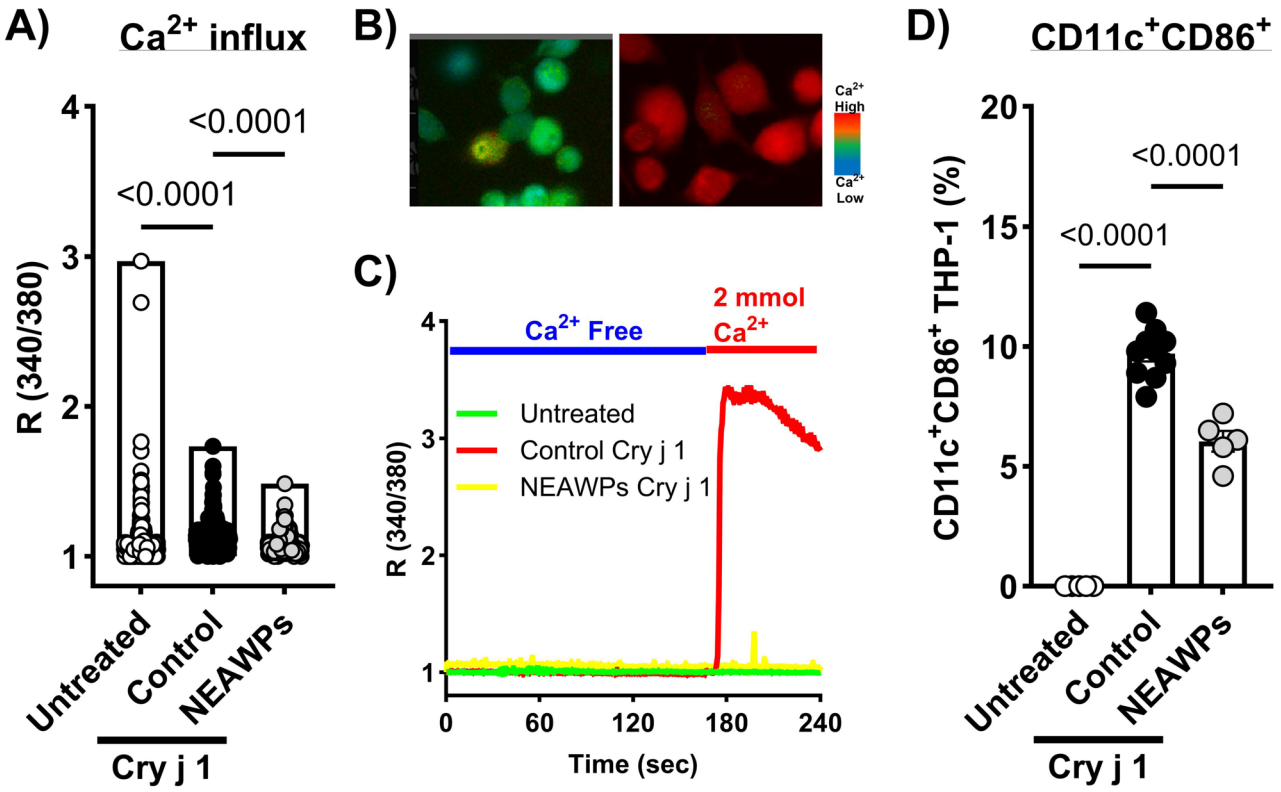


Fig. 2 Effect of NEAWP treatment on Cry j 1-induced activation of BEAS-2B. **A** Control Cry j 1 (0.5 μg/mL) significantly induced activation of BEAS-2B cells, whereas Cry j 1 pretreated with NEAWPs for 24 h markedly inhibited this activation. Calcium influx was measured using Fura-2-acetoxymethyl ester (%, mean ± SEM, n = 1201–1265). Representative (**B**) fluorescence images and (**C**) Ca^{2+} signals are shown. **D** Ratio of CD11c+CD86+ cells in THP-1 cells exposed to Cry j 1 or NEAWPs-treated Cry j 1 (mean ± SEM, n = 6)

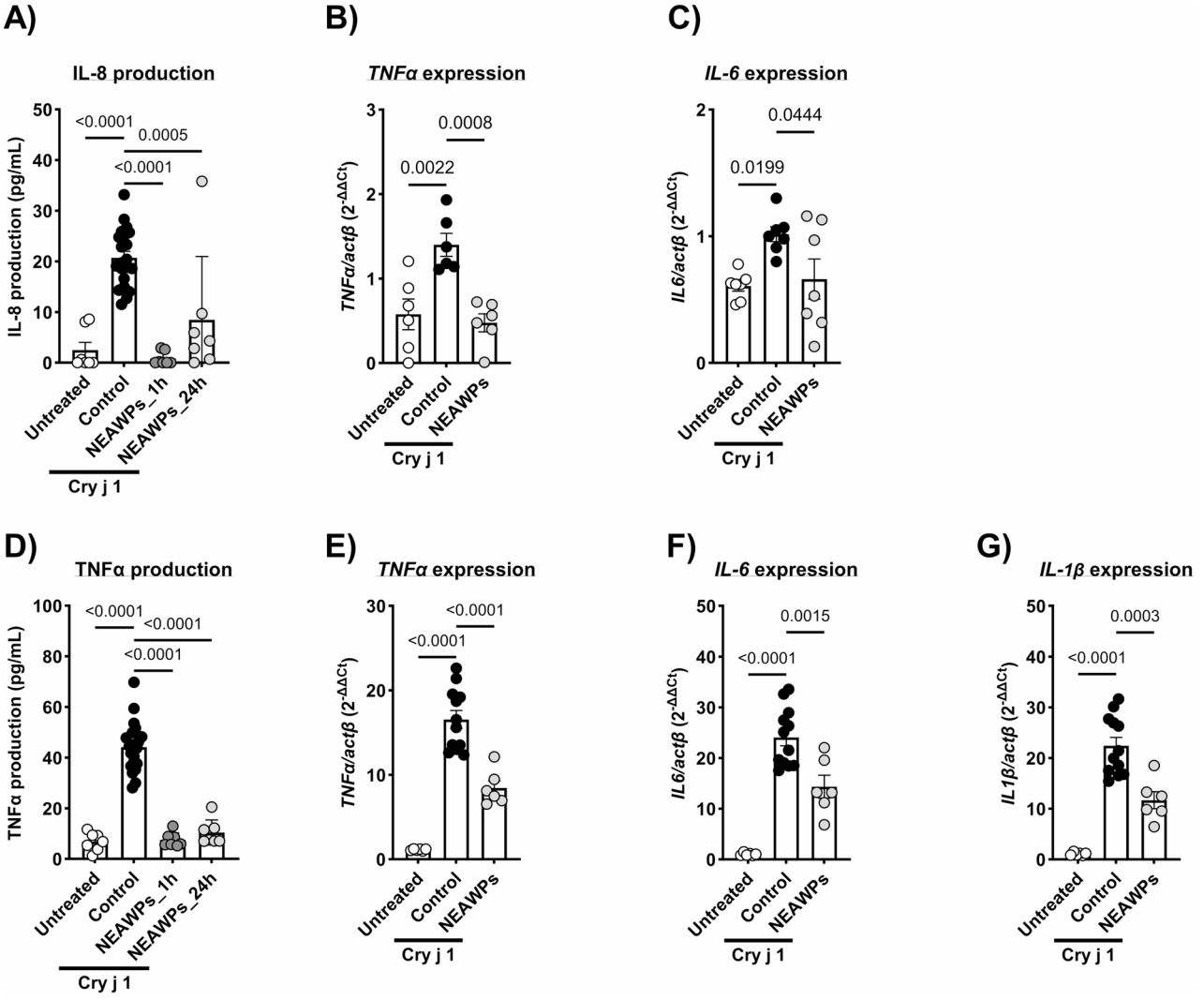


Fig. 3 Effect of NEAWP treatment on Cry j 1-induced proinflammatory cytokine production. (**A**–**C**) In BEAS-2B cells, NEAWP treatment significantly suppressed IL-8 protein production and reduced TNFα and IL-6 mRNA expression following Cry j 1 exposure. Panels **B** and **C** show results 24 h NEAWP treatment for 24 h. (**D**–**G**) In THP-1 cells, NEAWPs treatment significantly reduced TNFα protein production and decreased TNFα, IL-6, and IL-1β mRNA expression induced by Cry j 1. Panels **E**–**G** show results from 24 h NEAWPs treatment.

8 Discussion

In the present study, we evaluated the capacity of NEAWPs to inactivate Cry j 1 and reduce downstream allergic responses. NEAWPs are generated through the application of high-voltage electrostatic fields to condensed water droplets, which produces nanometer-scale particles containing ROS, such as hydroxyl radicals and superoxide anions. Previous studies have shown that NEAWPs inactivate a wide spectrum of biological targets, including viruses and bacteria, by inducing oxidative modification of their structural components. Here, we extend this concept to allergens, hypothesizing that NEAWPs may similarly denature allergenic proteins, thereby reducing their ability to elicit immune activation [12–15].

Our results confirmed that NEAWPs significantly reduced Cry j 1 antigenicity in a time-dependent manner, as demonstrated by ELISA. Specifically, 24 h exposure to NEAWPs reduced antigenicity by approximately 96%, and 48 h exposure resulted in nearly complete inactivation (98.6%). These findings align with previous studies demonstrating that NEAWPs exert potent oxidative effects on biological molecules and suggest that allergenic proteins are similarly susceptible to ROS-mediated denaturation. Given that allergenicity depends on the preservation of specific conformational and linear epitopes capable of cross-linking IgE bound to mast cells and basophils, partial denaturation may be sufficient to impair recognition and subsequent immune activation.

Airway epithelial cells represent the first barrier encountered by inhaled allergens and play a central role

in orchestrating immune responses [17–21]. Beyond their physical barrier functions, epithelial cells actively secrete cytokines and chemokines that recruit and activate downstream immune cells. For example, IL-8 recruits neutrophils, TNF-α promotes stromal and immune activation, and IL-6 contributes to inflammatory and tissue repair processes [22–26]. Consequently, excessive or prolonged cytokine secretion contributes to airway hyperresponsiveness, tissue remodeling, and the disruption of epithelial barrier integrity. Previous studies have shown that allergens, such as house dust mite proteins, activate protease-activated receptor-2 (PAR-2) and calcium channels, leading to intracellular Ca^{2+} influx and downstream cytokine release. Accordingly, in our experiments, BEAS-2B cells were initially perfused with a Ca^{2+}-free buffer, and extracellular Ca^{2+} was reintroduced at 180 s. This experimental design allows selective evaluation of Ca^{2+} influx across the plasma membrane rather than Ca^{2+} release from intracellular stores. Accordingly, control Cry j 1 stimulation induced a rapid increase in intracellular Ca^{2+} following Ca^{2+} readdition, indicating activation of Ca^{2+} entry pathways associated with epithelial cell activation. In contrast, NEAWPs-treated Cry j 1 failed to induce Ca^{2+} influx after extracellular Ca^{2+} reintroduction, demonstrating that NEAWPs treatment markedly impairs Cry j 1–mediated Ca^{2+} signaling. These findings suggest that oxidative modification of Cry j 1 by NEAWPs disrupts the molecular interactions required for allergen-induced Ca^{2+} channel activation, thereby attenuating early epithelial signaling events that contribute to downstream inflammatory responses. Moreover, Cry j 1 exposure significantly upregulated IL-8 secretion and increased TNF-α and IL-6 mRNA expression. Notably, NEAWP-treated Cry j 1 failed to induce such responses, and the Ca^{2+} influx and cytokine production returned to baseline levels comparable to untreated controls. These findings indicate that NEAWPs reduce antigenicity and abrogate allergen-induced epithelial signaling cascades. Therefore, mechanistically, we propose that the oxidative modification of Cry j 1 alters or destroys the key protein structures required for receptor interactions, thereby preventing downstream epithelial activation. With respect to exposure duration, NEAWPs treatment for 1 h was sufficient to markedly suppress Cry j 1–induced IL-8 production and other allergic responses in BEAS-2B cells, and no further reduction was observed following 24 h exposure. This suggests the presence of a threshold effect, whereby short-term NEAWPs exposure already achieves maximal inhibition of allergen-induced cytokine production. Importantly, prolonged exposure did not enhance allergic responses, indicating that NEAWPs treatment does not restore Cry j 1 allergenicity over time. The mechanisms underlying this apparent threshold effect, as well as its relevance under physiological conditions, remain to be elucidated and will be addressed in future studies using in vivo animal models.

Antigen-presenting cells such as dendritic cells and monocytes are essential for initiating adaptive immune responses. Upon allergen uptake, mainly $CD11c^{+}CD86^{+}$APCs process and present peptides via MHC class II molecules, leading to the activation of allergen-specific CD4 + T cells. This process is accompanied by the secretion of cytokines including IL-6, IL-1β, and TNF-α, which further shape the inflammatory environment and skew T cell differentiation toward Th2 responses [27–30]. Accordingly, we observed that Cry j 1 significantly activated and induced robust cytokine production in THP-1 monocytic cells. In contrast, NEAWP-treated Cry j 1 markedly suppressed these responses, suggesting that oxidative denaturation impairs antigen uptake and/or processing or reduces the availability of intact epitopes for MHC class II presentation. This suppression of APC activation has important implications. Theoretically, NEAWPs could reduce the initiation and amplification of Th2-mediated allergic responses by limiting antigen presentation, potentially dampening sensitization and subsequent effector phases of allergy.

The current findings extend previous research demonstrating the broad-spectrum antimicrobial properties of NEAWPs. Previous studies have shown that NEAWPs inactivate influenza virus, SARS-CoV-2, and *Escherichia coli* by inducing oxidative damage to surface proteins and nucleic acids. Our results indicate that a similar principle applies to non-infectious allergens that rely on intact protein epitopes to elicit immune responses. This highlights the fact that NEAWPs are unique interventions capable of simultaneously targeting infectious and allergenic agents. Allergen denaturation is not an unprecedented therapeutic approach. For instance, heat treatment, enzymatic digestion, and chemical modification have been explored as methods to reduce the allergenicity of foods and environmental allergens [31–33].

Although our study did not directly investigate the molecular mechanisms underlying Cry j 1 inactivation, previous studies have suggested several possibilities. ROS, such as hydroxyl radicals, are highly reactive and capable of inducing oxidative modifications in amino acid side chains, peptide backbone cleavage, and cross-linking [34, 35]. Such modifications can disrupt conformational epitopes that are critical for IgE recognition. Additionally, ROS can oxidize aromatic residues such as tryptophan and tyrosine, which often contribute to protein folding and stability. The cumulative effect of these alterations is likely due to denaturation of the allergenic protein and loss of its immunogenic capacity. Therefore, future studies employing mass spectrometry or structural

analyses are essential for confirming the specific modifications induced by NEAWPs in Cry j 1.

From a translational perspective, our findings suggest that NEAWPs can be used to prevent pollinosis. NEAWP-generating devices are used for air purification, and their ability to inactivate pathogens and allergens positions them as dual-function environmental interventions. Particularly for individuals with cedar pollinosis, continuous exposure to NEAWPs in indoor environments can reduce the allergen load, thereby alleviating symptoms or decreasing the risk of sensitization. Additionally, NEAWPs are produced from atmospheric water vapor without the need for chemical additives, making them environmentally friendly. Moreover, the integration of NEAWP technology into public spaces such as schools, offices, and transportation systems could have a broader public health impact. Thus, the ability of NEAWPs to simultaneously reducing airborne allergen and pathogen loads may contribute to improved respiratory health outcomes in populations with a high prevalence of allergic and infectious diseases.

Despite these promising findings, this study has some limitations that must be acknowledged. First, our experiments were conducted exclusively in vitro using monocultures of epithelial and monocytic cell lines. Although these models are useful for mechanistic exploration, they do not capture the complexity of in vivo airway environments, where multiple cell types, including fibroblasts, endothelial cells, and various immune cells, interact dynamically. Intercellular communication through cytokine networks and physical interactions is critical for shaping allergic responses. Consequently, future studies should employ co-culture systems or organoid models to better approximate physiological conditions. Second, system-level factors such as airflow patterns, residence time, and NEAWP concentration distribution were not addressed. These parameters are critical for translating biological inactivation into practical IAQ improvements and should be examined in future studies integrating engineering and environmental measurements. Third, although our data suggest that NEAWPs denature Cry j 1, we did not directly assess the structural modifications at the molecular level. In this regard, future studies using biophysical techniques, such as circular dichroism spectroscopy, differential scanning calorimetry, and high-resolution mass spectrometry, can provide direct evidence of protein unfolding, oxidation, or fragmentation. Such analyses are crucial for establishing the precise chemical modifications responsible for the reduced antigenicity. Third, our study did not evaluate in vivo outcomes. Therefore, whether NEAWP exposure reduces allergic sensitization or symptom severity in animal models and humans remains unclear. In vivo experiments using murine models of allergic rhinitis and asthma have provided critical evidence for the translational potential of this approach. Such studies could assess whether environmental exposure to NEAWPs reduces IgE production, airway hyper-responsiveness, and inflammatory cell infiltration following allergen challenge. Ultimately, clinical trials are necessary to determine the efficacy and safety of these drugs in humans. Finally, the long-term effects of the continuous inhalation of NEAWPs have not been fully characterized. Although prior studies have suggested minimal cytotoxicity, systematic toxicological evaluations are required to ensure safety before the widespread adoption of NEAWPs devices as preventive tools for allergic diseases.

In summary, this study provides the first evidence that NEAWPs reduce the allergenicity of Japanese cedar pollen by denaturing Cry j 1 and suppressing the subsequent immune activation in airway epithelial and antigen-presenting cells. Specifically, NEAWPs attenuate key steps in the allergic cascade by impairing early epithelial signaling and downstream antigen presentation. These findings expand our understanding of NEAWP biological activity and open new avenues for allergy prevention. Consequently, future studies should focus on elucidating the molecular mechanisms underlying allergen denaturation, validating its efficacy in in vivo models, and exploring its clinical applications. If confirmed, the NEAWPs represent a practical, environmentally friendly, and dual-purpose technology that is capable of simultaneously reducing airborne allergens and pathogens. Although the exposure durations used in this study exceed the expected contact time between airborne Cry j 1 and NEAWPs under real-world conditions, they were employed as a proof-of-concept approach to evaluate the intrinsic capacity of NEAWPs to chemically inactivate allergenic proteins. This approach has the potential to significantly improve the quality of life of individuals suffering from pollinosis and may provide a novel strategy for addressing the broader global burden of allergic diseases.

Supplementary Information

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Supplementary Material 1.

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Authors' Contribution

Sasa Iwamatsu: Conceptualization, Data curation, Investigation, Formal analysis, Methodology, Writing of the original draft. Yuta Kaga: Conceptualization, Data curation, Investigation, Formal analysis, Methodology, Writing of the original draft. Yasuhiro Komura: Conceptualization, Writing, reviewing, and editing. Yohei Ishigami: Conceptualization, Writing,

reviewing, and editing. Tomoki Fukuyama: Conceptualization, Data curation, Investigation, Formal analysis, Methodology, Writing the original draft.

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Data availability
The datasets generated and analyzed in the current study are available from the corresponding authors upon reasonable request.

Declarations

Competing interests
The authors declare the following competing financial interests: SI, YK, and YI were employed by Panasonic Corporation. The remaining authors declare no relevant financial or non-financial interests.

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