

Letter to the Editor

Environmental changes could enhance the biological effect of Hop J pollens on human airway epithelial cells

To the Editor:

Urbanization in Asian countries (including Korea and China) is rapidly increasing; subsequently, this urbanization has increased atmospheric temperatures and carbon dioxide concentrations, which affect the characteristics of plant pollens¹ and the prevalence of allergic diseases sensitive to pollens.² *Japanese hop* (Hop J) of the Hemp family (Cannabaceae) is considered a principle allergenic plant and its pollen is widespread during the autumn in Asian countries, particularly in Korea.³ Inhaled pollens can contribute to respiratory allergy symptoms by releasing several kinds of proteases that cause airway epithelium impairment and oxidases that induce the production of reactive oxygen species (ROS) in airway epithelial cells.^{4,5} Pollens can facilitate

the penetration of allergens through a disrupted epithelial barrier and augment allergic airway inflammation.^{4,5} We previously demonstrated that the allergenic potency of Hop J pollen has increased in the last 10 years in terms of serum-specific IgE response and the IgE-binding component of a major allergen at 10 kDa.³ In this study, we evaluated the effects of environmental changes on the biological effect of Hop J pollen extracts that can contribute to immune responses in human airway epithelial cells (AECs).

We collected Hop J pollens from Suwon, Gyeonggi province, South Korea, in 1998 and 2009 and prepared 2 pollen extracts (the 98 and 09 extracts) as described in the [Methods](#) section of this article's Online Repository at www.jacionline.org. The airborne Hop J pollen count and period of pollination has steadily increased over the last 10 years along with the average atmospheric temperature (0.07°C higher in 2000s than in 1990s in Suwon) and CO₂ concentration (21.8 ppm higher in 2009 than

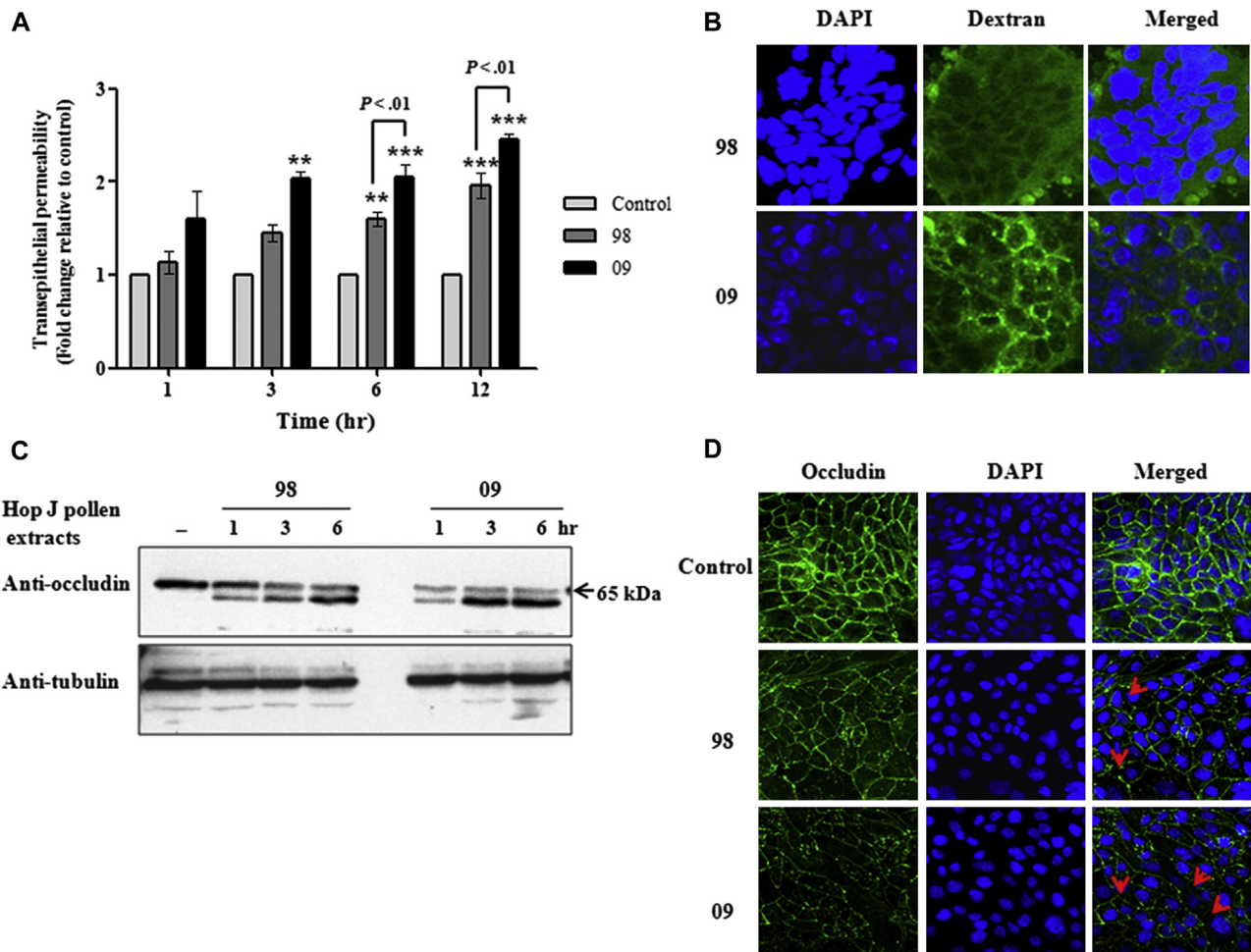


FIG 1. Effects of Hop J pollen extracts on transepithelial permeability and occludin degradation in airway epithelial cells. **A**, Transepithelial permeability of Calu-3 cells induced by the 2 pollen extracts. Data are shown as mean $\pm$ SEM. $^{**}P < .01$; $^{***}P < .001$ vs control. **B**, The disruption of tight junctions induced by the 2 pollen extracts visualized under a fluorescent microscope. **C**, Occludin degradations induced by Hop J pollen extracts. **D**, Integrity of occludin was visualized by immunocytochemistry. The arrows indicate examples of occludin degradation. Controls were conducted using serum-free media. DAPI, 4'-6-Diamidino-2-phenylindole, dihydrochloride; 98, the 98 extracts; 09, the 09 extracts.

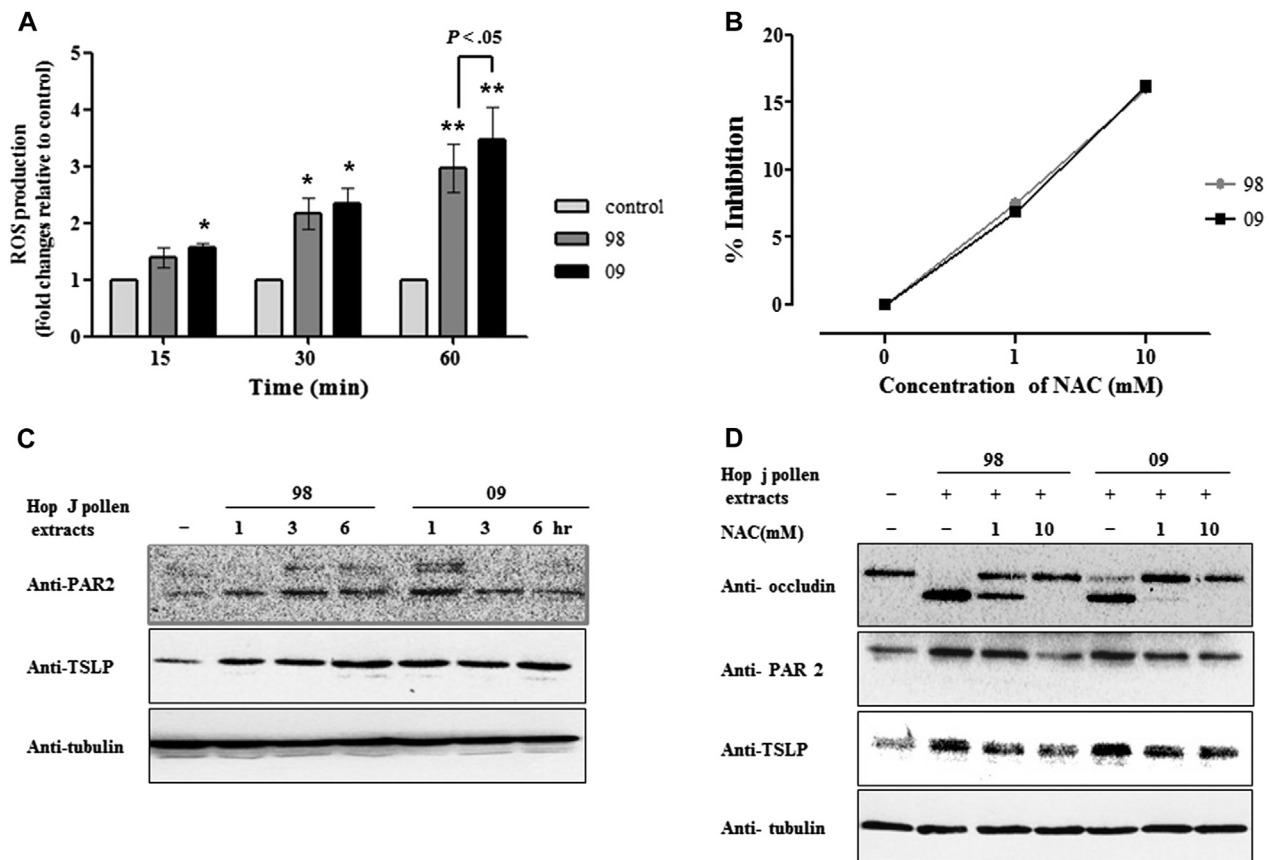


FIG 2. Hop J pollen extracts induced occludin degradation and upregulated PAR2 and TSLP expressions via ROS production. **A**, ROS production induced by the 98 and 09 extracts. Data are shown as mean $\pm$ SEM. $*P < .05$; $**P < .01$ vs control. **B**, NAC inhibited pollen-induced ROS productions. **C**, Expressions of PAR2 and TSLP upregulated by Hop J pollen extracts. **D**, NAC treatment inhibited the degradation of occludin as well as the upregulated expressions of PAR2 and TSLP induced by Hop J pollen extracts. Controls were conducted using serum-free media. 98, The 98 extracts; 09, the 09 extracts.

in 1999).³ The evidence suggests that Hop J pollen is sensitive to environmental change (particularly climate change), which could alter the bioeffects of Hop J pollen. Human AECs, Calu-3, were cultured using an air-interfaces method and treated with the 2 Hop J pollen extracts (100 μ g/mL) and fluorescein isothiocyanate-labeled dextran 70S (Sigma, St Louis, Mo) to evaluate the transepithelial permeability. Statistical analyses were performed using ANOVA followed by Bonferroni posttest. P values of less than .05 were considered statistically significant. The expression and integrity of occludin, a tight-junction protein, were investigated by Western blot and immunocytochemistry from pollen-treated cells. In Western blot results, the upper bands at 65 kDa represent the whole occludin molecules and the lower bands represent the cleaved domains of occludin molecules. Both 98 and 09 extracts induced increased transepithelial permeability (Fig 1, A and B) and the degradation of occludin (Fig 1, C and D) in Calu-3 cells time-dependently. In addition, the 09 extracts had significantly greater effects than the 98 extracts after 6 and 12 hours of incubation ($P < .01$ for transepithelial permeability induced by the 98 and 09 extracts). These findings suggest that environmental changes could augment proteolytic activity of Hop J pollen that can disrupt the epithelial tight-junction and enhance sensitization to exposed allergens.

Because pollen nicotinamide adenine dinucleotide phosphate oxidases could rapidly trigger ROS production and oxidative stress in AECs,⁵ we evaluated the Hop J pollen-induced ROS production by treating Calu-3 cells with the 98 and 09 extracts for 60 minutes followed by incubating for 30 minutes with 10 μ M of 2',7'-dichlorodihydrofluorescein diacetate. P values were obtained by ANOVA followed by Bonferroni posttest. We observed that both 98 and 09 extracts induced intrinsic ROS productions in Calu-3 cells time-dependently. In addition, a significantly higher ROS production was induced by the 09 extracts than by the 98 extracts after 60 minutes of incubation ($P < .05$) (Fig 2, A). To determine the effect of an antioxidant on Hop J pollen-induced ROS production in AECs, we treated Calu-3 cells with the 2 Hop J pollen extracts for 60 minutes in the presence of *N*-acetylcysteine (NAC), an ROS scavenger. The % inhibition of ROS production by NAC was calculated by using the following formula: [(ROS production without NAC treatment - ROS production with NAC treatment) $\times$ 100%]. Considerable inhibitions of the Hop J pollen-induced ROS productions by NAC were noted in a dose-dependent manner (Fig 2, B). Our previous study demonstrated a greater increased intensity of IgE-binding components in the 09 extracts than in the 98 extracts.³ These findings indicate that Hop J pollen exposure could induce airway inflammation via 2 mechanisms: ROS-mediated and allergen-mediated pathways.

Both these mechanisms have been intensified with the environmental changes.

Pollen proteases can activate pattern recognition receptors such as protease-activated receptors (PARs) expressed on human AECs.⁶ PAR2 has been implicated in airway inflammation by stimulating AECs to produce cytokines and chemokines and enhancing the responses of inflammatory cells.⁷ The activation of PAR2 in AECs by proteases results in the increased production and secretion of thymic stromal lymphopoietin (TSLP),⁸ a master mediator of T_H2-driven inflammation in asthma.⁹ In the present study, we observed that Hop J pollen extracts upregulated the expression of PAR2 and TSLP in human AECs. In addition, the 09 extracts (compared with the 98 extracts) induced slightly higher expressions of PAR2 as well as TSLP at 1 hour after stimulating (Fig 2, C). This finding suggests that Hop J pollen exposure could activate as well as amplify the PAR2-TSLP pathway that facilitates T_H2 immune responses in airway mucosa.

To determine whether Hop J pollen extracts upregulated PAR2 and TSLP expressions via ROS production, Calu-3 cells were treated with the 98 and 09 extracts for 6 hours in the presence of NAC. We observed that the expressions of PAR2 and TSLP returned to the baseline levels and the degradation of occludin recovered dose-dependently after NAC treatment (Fig 2, D). These findings suggest that Hop J pollen extracts could upregulate the expressions of PAR2 and TSLP as well as induce the degradation of occludin via ROS production. Moreover, NAC could be beneficial to suppress the development of sensitization and immune responses induced by Hop J pollens.

This is the first study to provide the evidence of occludin degradation and upregulated expressions of PAR2 as well as TSLP induced by Hop J pollen via pollen-induced ROS production; subsequently, the benefit of NAC to suppress T_H2-driven inflammatory responses to Hop J pollens was confirmed.

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METHODS

Hop J pollen extracts preparation

Hop J pollen was collected from Suwon, Gyeonggi province, South Korea, from mid-August to the end of September when Hop J pollen is the most prevalent, in 1998 and 2009 (the 98 and 09 extracts). The flowers were harvested directly from several weeds and air dried; subsequently, the pollens were dropped and collected through a sieve. The pollen then

was defatted with ethyl ether, dried, and extracted by stirring (24 hours at 4°C) in PBS. The mixtures were centrifuged at 2000 rpm for 30 minutes and the supernatants were collected and filtered through a 0.22- μ m filter (Millipore, Billerica, Mass). Protein concentrations of pollen extracts were measured by using a bicinchoninic acid assay (Bio-Rad, Hercules, Calif). Pollen extracts were stored at -70°C until required for experiments.