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Invited Review Article

Japanese cedar and cypress pollinosis updated: New allergens, cross-reactivity, and treatment

Toshihiro Osada^{a,*}, Mitsuhiro Okano^b^a Discovery and Preclinical Research Division, Taiho Pharmaceutical Co., Ltd., Ibaraki, Japan^b Department of Otorhinolaryngology, International University of Health and Welfare School of Medicine, Chiba, Japan

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ABSTRACT

Pollen from many tree species in the Cupressaceae family is a well-known cause of seasonal allergic diseases worldwide. Japanese cedar pollinosis and Japanese cypress pollinosis, which are caused by pollen from Japanese cedar (*Cryptomeria japonica*) and Japanese cypress (*Chamaecyparis obtusa*), respectively, are the most prevalent seasonal allergic diseases in Japan. Recently, the novel major Japanese cypress allergen Cha o 3 and the homologous Japanese cedar allergen Cry j cellulase were identified, and it was shown, for the first time, that cellulase in plants is allergenic. Although the allergenic components of pollen from both species exhibit high amino acid sequence identity, their pollinosis responded differently to allergen-specific immunotherapy (ASIT) using a standardized extract of Japanese cedar pollen. Pharmacotherapy and ASIT for Japanese cedar and cypress pollinosis have advanced considerably in recent years. In particular, Japanese cedar ASIT has entered a new phase, primarily in response to the generation of updated efficacy data and the development of new formulations. In this review, we focus on both Japanese cypress and cedar pollinosis, and discuss the latest findings, newly identified causative allergens, and new treatments. To manage pollinosis symptoms during spring effectively, ASIT for both Japanese cedar and Japanese cypress pollen is considered necessary.

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Japanese cedar and cypress pollinosis

Increasing dispersal of Japanese cedar and cypress pollen

Trees in the family Cupressaceae have a cosmopolitan distribution and are found on all continents except Antarctica; a total of 162 species in 32 genera and seven subfamilies have been described to date.¹ Pollen from several trees in the Cupressaceae, especially those in the genera *Cryptomeria*, *Chamaecyparis*, *Cupressus*, *Hesperocyparis*, and *Juniperus*, has been reported to cause allergic diseases. For example, pollen from mountain cedar (*Juniperus ashei*), eastern red cedar (*Juniperus virginiana*), and Arizona cypress (*Hesperocyparis arizonica*, formerly *Cupressus arizonica*) in the United States, as well as Italian cypress (*Cupressus sempervirens*) and prickly juniper (*Juniperus oxycedrus*) from the Mediterranean region, is known to cause seasonal allergic diseases. The amount of cypress pollen in Europe is increasing, and the incidence of cypress pollinosis is increasing accordingly.²

In Japan, the most widespread members of the family Cupressaceae are Japanese cedar (*Cryptomeria japonica*; “sugi” in Japanese), which belongs to the subfamily Taxodioideae, and Japanese cypress (*Chamaecyparis obtusa*; “hinoki” in Japanese) which belongs to the subfamily Cupressoideae (Fig. 1A). Pollen from the Japanese cedar is the major cause of seasonal allergic diseases in Japan, followed by pollen from Japanese cypress. The pollen grains of both species measure approximately 30 μm, but those of Japanese cedar have a characteristic papilla, while those of cypress pollen are almost spherical and can easily be distinguished from cedar pollen based on shape (Fig. 1B). The male flowers of Japanese cedar are generally larger than those of cypress (Fig. 1C). Dispersal of Japanese cedar pollen typically occurs from February to April, with a peak in March, while dispersal of Japanese cypress pollen typically extends from March to May, with a peak in April. Japan therefore has a unique situation in which pollen dispersal from these two species occurs over a period of more than 10 weeks, resulting in prolonged allergic symptoms due to allergenic similarities of the two species. Most Japanese cedar and cypress trees were planted as part of a national program that extended from the early 1950s to the early 1970s; as of 2017, approximately 20% of the entire Japanese land area is covered by artificial forests of Japanese

* Corresponding author. Discovery and Preclinical Research Division, Taiho Pharmaceutical Co., Ltd., 3 Okubo, Tsukuba, Ibaraki, 300-2611, Japan.

E-mail address: toshi-osada@taiho.co.jp (T. Osada).

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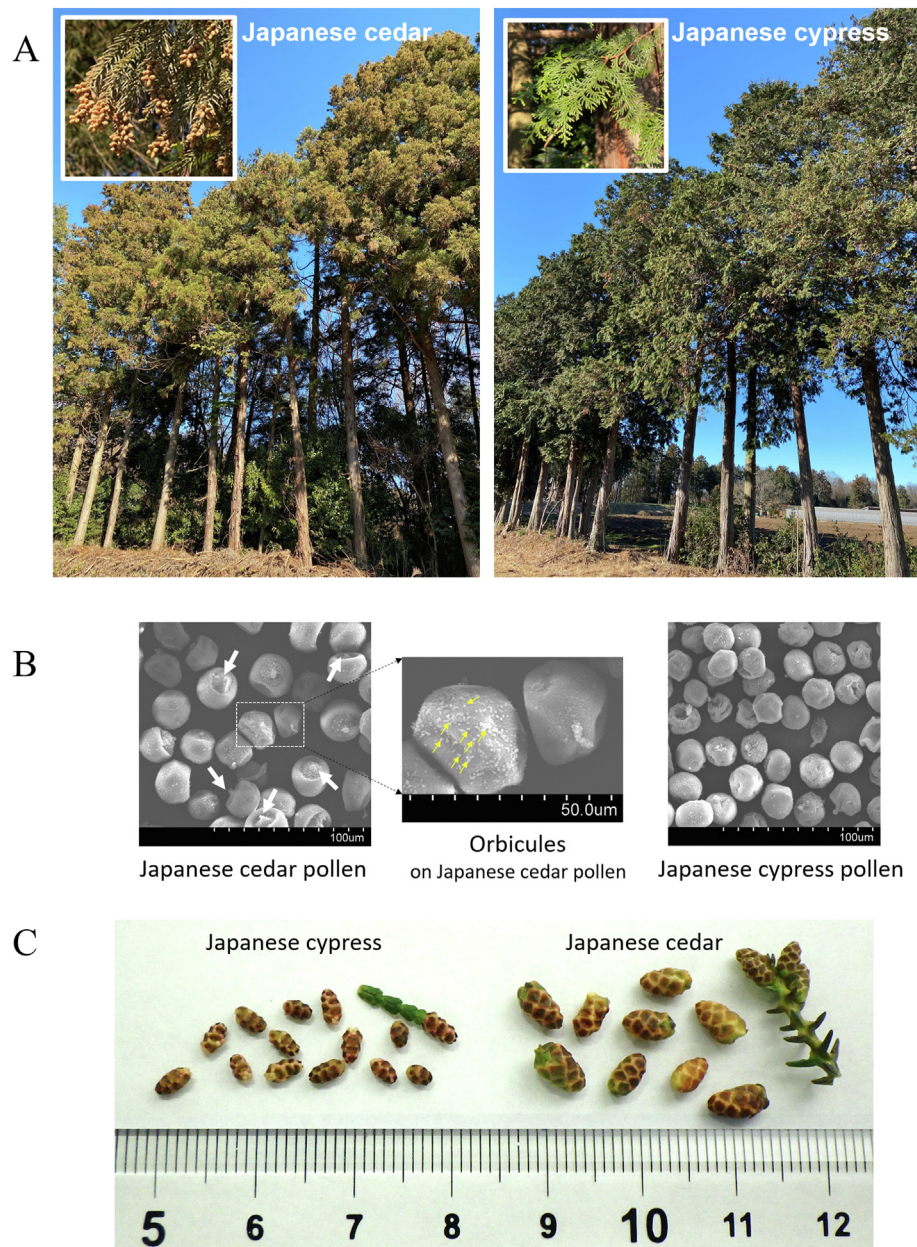


Fig. 1. Comparisons of Japanese cedar and Japanese cypress. **(A)** Japanese cedar (left) and Japanese cypress (right) trees. Pictures were taken on December 31, 2020 in Tsukuba city, Ibaraki, Japan. In this image, the Japanese cedar leaves appear brown in color (compared to those of Japanese cypress) because Japanese cedar pollen dispersal was imminent. Hence, the cedar trees were covered with numerous male flowers. Note that the shapes of leaves differ between the two species. **(B)** Scanning electron microscope images of Japanese cedar and Japanese cypress pollens. The white arrow indicates a papilla. Multiple orbicules (yellow arrow) are visible on the surfaces of both pollens. **(C)** Male flowers of Japanese cedar and Japanese cypress. Male flowers were harvested approximately 1 month before pollen dispersal. Male flowers from both species would typically continue growing until the initiation of pollen dispersal. The scale on the ruler is in millimeters.

cedar and cypress trees.³ Japanese cedar is widely distributed throughout Japan, and Japanese cypress is especially prevalent in western Japan. Similar to cypress trees in Europe and the United States, Japanese cedar and cypress pollen counts continue to increase each year. For example, Japanese cedar pollen counts in the mountainous areas of Akita Prefecture in northeastern Japan almost doubled between 1996 and 2006.⁴ Kishikawa *et al.* reported the results of a continuous survey of Japanese cedar and cypress pollen counts at nine sites throughout Japan from 1987 to 2013. Their results clearly showed a sustained increase in amounts of pollen from Japanese cedar and cypress nationwide.⁵ In Oita Prefecture, located in Kyushu Island, the results of a survey from 1996 to 2017 showed significant increases in both cedar and cypress

pollen counts.⁶ There are several possible reasons for the recent increase in Cupressaceae pollen counts in Japan. The major reason is that the Cupressaceae trees that were planted in the 1970s are now approaching mature and their pollen production has increased. Additionally, since pollen production by Japanese cedar have been reported to be positively correlated with the previous summer's average temperature,^{7,8} the increase in pollen counts could also be ascribed to global warming.^{9,10} Furthermore, although this phenomenon has not been fully discussed to date, we are particularly concerned about recent increases in the quantities of pollen produced by Japanese cypress. In fact, the count (or the rate of increase in the count) of Japanese cypress pollen sometimes exceeds that of cedar pollen in some areas.^{5,11,12} Surprisingly,

although the precise causes are unclear, cypress pollen counts in the Kanto region, which has a relatively small area under cypress forest, are higher than those in the more-heavily forested Kyushu region in western Japan.⁷ The Kanto region, which includes Tokyo, is the most densely populated region in Japan, accounting for 33.8%¹³ of the total population of the country (as of 2015). There is therefore some concern that such massive exposure to Japanese cypress pollen might result in an escalation in the sensitization to Japanese cypress allergens and pollinosis in the near future.

Prevalence of Japanese cedar/cypress pollinosis

It has been reported that Japanese cedar pollen counts are correlated with the rate of sensitization to the cedar pollen allergen, and consequently, that the prevalence of Japanese cedar pollinosis is increasing along with pollen counts.⁴ In a study on Jeju Island, the only site in Korea with a substantial Japanese cedar pollen count, exposure to Japanese cedar pollen for more than two seasons elevated the risk of sensitization; longer residence times significantly increased the positive rate of skin prick tests.¹⁴ Other recent reports indicate that pollen exposure at a young age is particularly likely to trigger the onset of pollinosis. In fact, a significant increase of Japanese cedar pollinosis has been observed among 5- to 19-year-olds.¹⁵ Similarly, in a comparison of Japanese residents born in March, when Japanese cedar pollen dispersal is at its peak, those born after 1973 were found to be at higher risk of developing Japanese cedar pollinosis than those born before 1973 when cedar pollen dispersal was less common.¹⁶ A follow-up study among 5- to 9-year-olds ($n = 651$) in Tokyo showed that the rate of Cry j 1-specific IgE positivity was already 32.8% in 5-year-olds and that it increased to 57.8% over the following 4 years.¹⁷

The prevalence of the disease in all age groups also is increasing. According to the results of an epidemiological survey of Japanese cedar pollinosis conducted in 2001, the estimated prevalence of cedar pollen allergy was 13.1%.¹⁸ In 1998 and 2008, Baba *et al.* reported the results of a nationwide study on the prevalence of allergic rhinitis among otolaryngologists and their families in Japan.¹⁹ In 2019, Matsubara *et al.* used the same questionnaire in a survey of 19,859 Japanese residents to assess changes in the prevalence of allergic rhinitis over the preceding decade. Surprisingly, the prevalence of Japanese cedar pollinosis increased from 16.2% in 1998 to 26.5% in 2008 and 38.8% in 2019, while the prevalence of pollinosis other than Japanese cedar pollinosis increased from 10.9% to 15.4% and 25.1%, respectively, revealing that the prevalence of pollinosis (both cedar-specific and other) continues to increase.¹⁵ These observations suggest that the prevalence of allergy to Cupressaceae pollen in Japan is extremely high and getting worse. For comparison, the prevalence of cypress pollen allergies is 3.6% in the Mediterranean region, where this allergy is considered common.²⁰

Interestingly, the prevalence of pollinosis caused by pollens other than Japanese cedar exceeded the perennial allergy prevalence (24.5%) in 2019.¹⁵ In addition to birch and grass pollinosis, the aforementioned increase in Japanese cypress pollen counts has presumably played a large role in this reversal. For example, a 17-year survey conducted in Hiroshima showed an increase in the rate of Japanese cypress allergen sensitization, while the rate of Japanese cedar pollen sensitization remained unchanged.²¹ While the increased prevalence of Cupressaceae pollinosis in Japan is considered to be a serious social problem, the situation has not improved and is getting worse.

With regard to their symptoms, although both Japanese cedar pollinosis and Japanese cypress pollinosis share rhinoconjunctivitis caused by type I allergic reactions, it has been reported that throat symptoms, in particular, are more severe in Japanese cypress pollinosis, suggesting pathological differences between cedar and cypress pollinosis.²²

Allergens of pollen from Japanese cedar and Japanese cypress and their cross-reactivity

Cupressaceae pollen allergens consist of several allergen groups across tree species, with high amino acid sequence identity and shared protein functions within each allergen group. Table 1 summarizes Cupressaceae pollen allergens identified from Cupressaceae pollen worldwide. In the sections below, we describe what is currently known about the pollen allergens from Japanese cedar and Japanese cypress and their cross-reactivity. It is well known that the pollen allergens of Japanese cedar exhibit cross-reactivity with the pollen allergens of Japanese cypress; indeed, approximately 70% of cedar pollinosis patients experience rhinitis symptoms during the Japanese cypress pollen season.¹⁰ Next, we will discuss the differences between Japanese cedar and cypress pollen allergens, a topic that has not received much attention to date.

Group-1 allergens: Cry j 1 and Cha o 1 (pectate lyase)

Cry j 1, the first major allergen to be identified from Japanese cedar pollen, was discovered in 1983.²³ Mature Cry j 1 is a 374-amino-acid protein with a molecular weight of approximately 38.5 kDa.²⁴ Cry j 1 is a glycoprotein with pectate lyase activity,²⁵ and the glycoform of the protein has been determined.²⁶ On SDS-PAGE, the protein runs as two bands with molecular weights of 45 and 50 kDa, likely due to the heterogeneity of glycosylation. The majority of Cry j 1 is localized on the pollen surface orbicules (Fig. 1B) and the outer membrane,²⁷ with a reported content of approximately 300–500 µg/g pollen.²⁸ Since Cry j 1 is highly allergenic, Cry j 1-specific immune responses can frequently be

Table 1
Cupressaceae pollen allergens.

Species	common name	Group 1	Group 2	Group 3	Cellulase	GRP	Calcium binding protein	Other allergens
<i>Cryptomeria japonica</i>	Japanese cedar	Cry j 1	Cry j 2	Cry j 3	Cry j cellulase	Cry j 7		CJP-4, CJP-6, CJP-8, CPA9, CPA63, CJP38
<i>Chamaecyparis obtusa</i>	Japanese cypress	Cha o 1	Cha o 2		Cha o 3			
<i>Juniperus ashei</i>	mountain cedar	Jun a 1	Jun a 2	Jun a 3		Jun a 7		
<i>Juniperus virginiana</i>	eastern red cedar	Jun v 1		Jun v 3				Jun v 4
<i>Hesperocyparis arizonica</i> (<i>Cupressus arizonica</i>)	Arizona cypress	Cup a 1	Cup a 2	Cup a 3			Cup a 4	β-Galactosidase
<i>Cupressus sempervirens</i>	Italian cypress	Cup s 1	Cup s 2	Cup s 3		Cup s 7		Cypmaclein, BP14, β-Galactosidase
<i>Juniperus oxycedrus</i>	prickly juniper	Jun o 1					Jun o 4	

GRP, gibberellin-regulated protein.

observed at both the T and B cell levels in Japanese cedar pollinosis patients, suggesting that this protein is the major allergen of Japanese cedar pollen. Hirahara *et al.* reported Cry j 1-induced T cell proliferation in 104 of 113 subjects (92%) exhibiting allergic symptoms during the Japanese cedar pollen season.²⁹ More recently, we observed positivity in the Cry j 1-specific lymphocyte stimulation test in 13 of 15 cases (87%).³⁰ Other studies have reported that 12 of 14 Japanese cedar pollinosis patients (86%) were seropositive for Cry j 1-specific IgE,³¹ and that 86% of Japanese patients (n = 28) with cedar pollen allergy were positive for Cry j 1-specific IgE.³² Given that IgE binding to Cry j 1 is strongly inhibited by heating (which would induce a conformational change in Cry j 1), it appears that the majority of the Cry j 1-specific IgE recognizes conformational epitopes,³³ although sequential IgE epitopes have also been identified.³⁴

In Japanese cypress pollen, the allergen Cha o 1 was identified as being homologous to Cry j 1.³⁵ Cha o 1 also is a glycoprotein comprising 374 amino acids,³⁶ and is also observed as two bands with molecular weights of 45 and 50 kDa by SDS-PAGE. The amino acid sequence of Cha o 1 is 78% identical to that of Cry j 1 (Table 2), and Cha o 1 is presumed to have pectate lyase activity similar to that of Cry j 1.³⁵ Both Cry j 1 and Cha o 1 are known to have high homology with pectate lyase allergens of other Cupressaceae pollens from around the world (Table 2). For example, the amino acid sequence of Jun a 1, which is a major pollen allergen of mountain cedar, has 78% sequence identity with Cry j 1.³⁷

The major T cell epitopes shared by Cha o 1 and Cry j 1 have been identified, along with T cell epitopes that are unique to Cha o 1.³⁸ Cry j 1 and Cha o 1 have been reported to exhibit cross-reactivity with each other at the IgE level.^{28,39} This cross-reactivity presumably reflects the presence of shared, or very similar, B cell epitopes due to the high amino acid sequence identity of the two allergens. However, a recent study showed that the cross-reactivity of anti-Cry j 1-specific IgE (cedar) to Jun a 1 and Cup a 1 (cypresses) was limited compared to that observed between Jun a 1 and Cup a 1. Such a finding would be consistent with the lower amino acid sequence identity between Cry j 1 and Jun a 1 (78%) compared to that between Jun a 1 and Cup a 1 (94%),³² prompting Pichler *et al.* to conclude that Cry j 1 belongs to a category other than Jun a 1/Cup a 1 at the level of IgE recognition.³² Notably, the findings of that study clearly illustrated differences in the immune response between cedar and cypress, which presumably reflects differences in sequences and/or allergen structure. Although there is no doubt that IgE cross-reactivity exists between Cry j 1 and Cha o 1, the importance of the presence of unique IgEs against each allergen should not be ignored.⁴⁰

Group-2 allergens: Cry j 2 and Cha o 2 (polygalacturonase)

Cry j 2 was the second major allergen from Japanese cedar pollen to be identified.^{31,41} Cry j 2 comprises 514 amino acids, and the protein is considered to be a polygalacturonase based on

sequence similarity to other plant polygalacturonases; however, only polymethylgalacturonase activity has been demonstrated experimentally.⁴² Cry j 2 is observed as a single band with a molecular weight of 37.5 kDa by SDS-PAGE under reducing conditions. Cry j 2 localizes in the starch grains of mature pollen and was initially considered to be less abundant than Cry j 1.⁴³ However, a subsequent report using an improved extraction method revealed that Cry j 2 is present at levels of approximately 400 µg/g pollen.⁴⁴ Cry j 2 also has a high sensitization rate among Japanese cedar pollinosis patients, with 71% of subjects exhibiting Cry j 2-specific IgE positivity.³¹ By using an epitope mapping strategy, both conformational and sequential IgE epitopes have been identified in humans.^{45,46} With regard to T cell sensitization, PBMCs from 111 of 113 subjects (98%) showed Cry j 2-specific T cell proliferation.²⁹

Cha o 2 from Japanese cypress pollen has been identified as a group-2 allergen.^{47,48} Cha o 2 is expressed as a 514-amino-acid pro-protein, including a 50-mer signal peptide, and mature Cha o 2 runs as an approximately 42- to 45-kDa band on SDS-PAGE. It has been reported that 82.5% of patients with Japanese cypress pollinosis have Cha o 2-specific IgE antibodies.⁴⁷ This group-2 allergen also is highly homologous to polygalacturonases found in Cupressaceae pollen from around the world (Table 3). The amino acid sequence of Cha o 2 has 74% identity with Cry j 2 (Table 3). In addition to possessing major T cell epitopes, which is shared with Cry j 2 (based on amino acid sequence identity),⁴⁹ Cha o 2 also exhibits IgE cross-reactivity with Cry j 2.⁴⁷ According to recent advances in group-2 allergen-specific IgE research, the antibody recognizing the Cry j 2 169-KVVNGREI-176 epitope was found to cross-react with the amino acid sequence 169-KVVNGRTV-176 in Cha o 2.⁵⁰ Conversely, as was seen with group-1 allergens, multiple major T cell epitopes that were unique to Cha o 2 have also been discovered.⁴⁹

Group-3 allergen: Cry j 3 (thaumatin-like protein [TLP] in PR-5 family)

The Cry j 3-encoding gene was cloned as a homolog of the locus encoding Jun a 3,⁵¹ which is a mountain cedar pollen allergen⁵²; the Cry j 3 protein was then subsequently isolated from Japanese cedar pollen.⁵³ The Cry j 3 protein consists of 223 amino acids and has 85% sequence identity with Jun a 3, which belongs to a family of TLPs (pathogenesis-related [PR]-5 family). TLPs have been identified as allergens in pollens from other Cupressaceae trees (Table 4), with high amino acid homology observed among the group-3 allergens. However, to date, a group-3 allergen homolog has not been identified from Japanese cypress pollen. The frequency of Cry j 3-specific IgE in Japanese cedar pollinosis patients has been reported to be 27% (27/100), suggesting that Cry j 3 is a minor allergen compared to other allergens. Cup a 3, a member of the group-3 allergens, has been reported to have cross-reactivity with TLP allergens in several fruits, suggesting an association between oral allergy syndrome (OAS) and group-3 allergens, including Cry j 3.⁵⁴

Table 2
Amino acid sequence identity among Cupressaceae group 1 allergens (pectate lyase).

allergen	Species	Cry j 1	Cha o 1	Jun a 1	Jun v 1	Cup a 1 [†]	Cup s 1
Cry j 1	<i>Cryptomeria japonica</i>	100%	78%	78%	78%	79%	77%
Cha o 1	<i>Chamaecyparis obtusa</i>	78%	100%	85%	85%	84%	83%
Jun a 1	<i>Juniperus ashei</i>	78%	85%	100%	96%	94%	95%
Jun v 1	<i>Juniperus virginiana</i>	78%	85%	96%	100%	94%	96%
Cup a 1	<i>Hesperocyparis arizonica</i> (<i>Cupressus arizonica</i>)	79%	84%	94%	94%	100%	95%
Cup s 1	<i>Cupressus sempervirens</i>	77%	83%	95%	96%	95%	100%

The sequence identity was re-analyzed using amino acid sequence data in the NCBI protein database. Accession numbers are as follows. Cry j 1, BAA05542; Cha o 1, BAA08246; Jun a 1, AAD03608; Jun v 1, AAF80166; Cup a 1, CAB62551; Cup s 1, AAF72625 (unpublished).

[†] Mature protein (signal peptide including the N-terminal methionine is unidentified).

Table 3

Amino acid sequence identity among Cupressaceae group 2 allergens (polygalacturonase).

allergen	Species	Cry j 2	Cha o 2	Jun a 2
Cry j 2	<i>Cryptomeria japonica</i>	100%	74%	68%
Cha o 2	<i>Chamaecyparis obtusa</i>	74%	100%	79%
Jun a 2	<i>Juniperus ashei</i>	68%	79%	100%

The sequence identity was reanalyzed using amino acid sequence data obtained from the NCBI protein database. Accession numbers are as follows: Cry j 2, BAA06172; Cha o 2, Q7M1E7; Jun a 2, CAC05582.

Reliable amino acid sequence data for Cup a 2 and Cup s 2 have not yet been deposited.

Table 4

Amino acid sequence identity among Cupressaceae group 3 allergens (Thaumatococcus-like protein).

allergen	Species	Cry j 3	Jun a 3	Cup s 3
Cry j 3	<i>Cryptomeria japonica</i>	100%	85%	84%
Jun a 3	<i>Juniperus ashei</i>	85%	100%	95%
Cup s 3	<i>Cupressus sempervirens</i>	84%	95%	100%

The sequence identity was re-analyzed using amino acid sequence data obtained from the NCBI protein database. Accession numbers are as follows: Cry j 3, nucleotide sequence AB254807 was translated to amino acid; Jun a 3, AAF31759; Cup s 3, AAR21073 (Unpublished).

Whole amino acid sequence data of Jun v 3 and Cup a 3 have not yet been deposited.

Newly identified group allergens: Cry j cellulase and Cha o 3 (cellulase family)

Our group recently identified a new major Japanese cypress pollen allergen, Cha o 3. Given that the amino acid sequence of Cha o 3 is completely different from those of group-3 allergens, Cha o 3 does not belong to the TLP family despite being designated as a No. "3" allergen. Cha o 3 comprises 556 amino acids, and mature Cha o 3 has a molecular weight of approximately 59 kDa.⁵⁵ Cha o 3 content was reported to be approximately 75 µg/g cypress pollen without optimization of the extraction method.⁵⁶ By SDS-PAGE, Cha o 3 is observed as two bands, with molecular weights of 63 and 66 kDa. Cha o 3 is a glycoprotein that possesses a unique glycosylation variation.⁵⁷ The protein has a cellulase (glycosyl hydrolase family 5) domain, and was shown to possess cellulolytic activity.⁵⁵ Although the function of plant allergens is known to be relatively limited,⁵⁸ Cha o 3 is the first example of a plant cellulase acting as an allergen. The allergenicity of Cha o 3 is as high as that of group-1 and group-2 allergens. Among patients with Japanese cedar and cypress pollinosis, 87.5% were positive for Cha o 3-specific IgE.⁵⁵ Furthermore, all of the patients in that study tested positive in Cha o 3-specific lymphocyte stimulation tests.⁵⁵ Cha o 3-specific IgE antibody titer was significantly correlated with the Japanese cypress ImmunoCAP results, and the addition of Cha o 3 to the sera of Japanese cypress pollinosis patients substantially reduced the Japanese cypress ImmunoCAP detection.^{30,55} These findings indicated that Cha o 3-specific IgE also plays a crucial role in the clinical test for the diagnosis of Japanese cypress pollinosis, even though the clinical test was primarily believed to be quantifying the binding by Cha o 1- and Cha o 2-specific IgE (and cross-reactive Cry j 1-/Cry j 2-specific IgE). Recently, the method used to produce the Japanese cypress ImmunoCAP test was improved to enhance allergen-specific-IgE-detection sensitivity.⁵⁹ Therefore, the contribution of Cha o 3-specific IgE to this new reagent will need to be re-investigated.

In 2018, Cry j cellulase was identified from Japanese cedar pollen and reported as being a Cha o 3-homologous allergen.³⁰ Cry j cellulase is a 552-amino-acid protein that possesses a cellulase domain and shows high amino acid identity (85%) to Cha o 3,

suggesting that Cry j cellulase also belongs to the cellulase family. Cross-reactivity of T cells and antibodies between Cha o 3 and Cry j cellulase was noted. Specifically, the presence of a major T cell epitope with an identical amino acid sequence was identified in both Cry j cellulase and Cha o 3, further supporting the cross-reactivity between these two allergens at the T cell level.⁵⁶ However, T cell clones specific to Cha o 3 also were identified, indicating differences in T cell responses between Japanese cedar and cypress pollen allergens similar to other major allergens.⁵⁶ Interestingly, the content of Cry j cellulase in Japanese cedar pollen is considerably lower than that of Cha o 3 in Japanese cypress pollen.³⁰ It is therefore possible that cellulase might be a dominant allergen, especially in members of the Cupressoideae subfamily (cypress) compared to those of the Taxodioideae subfamily (cedar). Although cellulase is essential for fertilization, providing degradation of the cellulose-rich cell wall of pollen during pollination, Cha o 3-homologous allergens have not been reported from other Cupressaceae trees. However, several studies have shown that an unknown IgE-reacting protein, approximately 70 kDa in size, was frequently observed in crude extractions of Arizona cypress and Common cypress pollen, suggesting the presence of cellulase family allergens.^{60,61}

Other allergens

Several other allergens have been identified or isolated from Japanese cedar pollen, including the class-IV chitinase, CJP-4⁶²; isoflavone reductase-like protein, CJP-6⁶³; lipid transfer protein homolog, CJP-8⁶⁴; subtilisin-like serine protease, CPA9⁶⁵; aspartic protease, CPA63⁶⁶; and β-1,3-glucanase, CJP38⁶⁷; all of which were shown to have considerable sensitization rates, exhibiting IgE positivity in patients of 100%, 76%, 37.5%, 88.5%, 58%, and 75%,⁶⁸ respectively (Table 1). Recently, Cup s 7, Jun a 7, and Cry j 7 were identified as homologs of Pru p 7, a peach allergen belonging to a family of gibberellin-regulated protein (GRP).⁶⁹ The Cry j 7 amino acid sequence is 94% identical to that of Cup s 7 and 92% identical to that of Jun a 7, although the identity to Pru p 7 is considerably lower (68%). Fruit allergy associated with cross-reactivity to pollen allergens is called pollen-food allergy syndrome (PFAS). The relationship between Cry j 7 sensitization and Pru p 7 sensitization appears to be associated with severe fruit allergy,⁷⁰ and therefore deserves further clinical investigation. To date, homologs of the allergens described in this section have not been identified from Japanese cypress pollen. Further experiments are therefore required in order to address the role of these allergens in Japanese cypress pollinosis.

Updated treatments for Japanese cedar/cypress pollinosis

Intriguingly, a 10-year follow-up study of the same patient cohort (n = 334) revealed that the spontaneous remission rate of Japanese cedar pollinosis was low (12.7%) compared to that of perennial allergic rhinitis (36.2%).⁷¹ In order to improve the quality of life (QOL) of Japanese cedar/cypress pollinosis patients, it will be necessary to actively develop new treatments. In the following sections, we summarize treatment options that have recently been developed for patients with seasonal allergic rhinitis (SAR), including Japanese cedar/cypress pollinosis.

Pharmacotherapy

Pharmacotherapy for Japanese cedar/cypress pollinosis includes mast cell stabilizer, antihistamines, antileukotrienes, nasal or oral steroids, anti-prostaglandin D₂/thromboxane A₂ (ramatroban), Th2 cytokine inhibitor (suplatast tosilate), and vasoconstrictors. Among these treatments, second-generation antihistamines are

recommended (by the 2020 Japanese guidelines for allergic rhinitis) for the treatment of Japanese cedar and cypress pollinosis, regardless of severity and disease type, such as sneeze/rhinorrhea type and congestion type.⁷² Since 2015, three new oral antihistamines and one antihistamine with altered drug delivery have been made available as treatment options for SAR in Japan. Bilastine is a non-sedating antihistamine with inverse agonistic activity that lacks the impaired performance associated with H1 receptor occupancy in the central nervous system (CNS); this compound has been shown to be effective against Japanese cedar pollinosis.^{73–75} In terms of downregulation of the H1 receptor, an antihistamine with inverse agonistic activity would be preferable for use in early interventional treatment for pollinosis.⁷⁶ Desloratadine also is a non-sedating antihistamine with inverse agonistic activity that demonstrates therapeutic efficacy in patients with Japanese cedar pollinosis.^{77,78} However, the findings of a recent study suggest that the inverse agonist activity of desloratadine is controversial.⁷³ Rupatadine is a non-sedating antihistamine with platelet-activating factor (PAF) receptor antagonism; this compound demonstrates allergic symptom suppression in patients with Japanese cedar pollinosis.^{79,80} At the recommended dose of 10 mg, rupatadine did not affect driving performance, but dose-dependent psychomotor impairments at higher doses of rupatadine were observed in healthy volunteers.^{81–83} Presumably, to be safe, care should be taken regarding CNS adverse events when escalating the rupatadine dose to 20 mg. Emedastine patch, the world's first transdermal antihistamine, was approved for use in Japan in 2018. Since this drug delivery system enables blood concentrations to be maintained within a therapeutic range, it has been shown to suppress SAR symptoms, especially sneezing and rhinorrhea, with a once-daily application providing a new treatment option with good adherence.⁸⁴

Since several studies have shown the clinical benefits and the mechanism of early interventional treatment with antihistamines, it is desirable to initiate antihistamine treatment around the start of continuous Japanese cedar pollen dispersal or at the onset of symptoms.^{85,86} However, it has been reported that early intervention with antihistamines has limitations for the treatment of Japanese cypress pollinosis when the dispersal of Japanese cypress pollen is particularly heavy.¹¹ Treatment of Japanese cedar/cypress pollinosis by early intervention with nasal corticosteroids was shown to reduce symptom scores significantly via suppression of minimal persistent inflammation, and efficacy (especially against sneezing) was increased with longer prophylactic treatment.^{87–89} Likewise, early intervention involving a 4-week course of antileukotrienes has been noted as being beneficial for both Japanese cedar and Japanese cedar/cypress pollinosis.^{90,91} Based on these findings, combined therapy with antileukotrienes and/or intranasal corticosteroids has been proposed for inadequately controlled cypress pollen allergies.¹¹ Most recently, omalizumab, a humanized anti-IgE monoclonal antibody, was approved for treatment of inadequately controlled severe or severest SAR, based on the data from a phase 3 clinical study.⁹² In that study, treatment with omalizumab, in combination with antihistamines and nasal corticosteroids, significantly improved nasal and ocular symptoms compared to administration of a placebo combined with antihistamines and nasal corticosteroids. Interestingly, this significant effect was not only seen in the cedar dispersal season, but also in the cypress dispersal season. These recent findings are expected to lead to a new era in the treatment of patients with Japanese cedar/cypress pollinosis.

Allergen-specific immunotherapy (ASIT)

ASIT is the only treatment that can fundamentally change the natural course of allergic disease and result in a long-term remission of allergic symptoms. In Japan, Japanese cedar pollen allergen-specific

subcutaneous immunotherapy (SCIT) using standardized Japanese cedar pollen extracts has been the subject of active development, providing significant improvement of symptom scores and QOL in patients with Japanese cedar pollinosis compared to pharmacotherapy alone.⁹³ However, the risk of systemic reaction, including anaphylaxis, has prevented the widespread adoption of ASIT. In 2014, a new standardized Japanese cedar pollen extract drop for sublingual immunotherapy (SLIT) was approved by the Pharmaceuticals and Medical Devices Agency (PMDA), making ASIT safer and easier relative to conventional SCIT for Japanese cedar pollinosis.⁹⁴ Furthermore, a clinical trial of Japanese cedar pollen SLIT tablets with a 2.5-fold increase in the Cry j 1 dosage (from 2000 Japanese allergy units [JAU] to 5000 JAU/day as a maintenance dose) effectively suppressed total nasal symptom medication scores (TNSMS) for three years.⁹⁵ Physicians began to switch from drops to tablets following the approval of the SLIT tablet. The therapeutic effect was shown to be dependent on the treatment duration, indicating that the treatment needs to be administered as continuously as possible.⁹⁶ The Japanese cedar SLIT tablet was approved for use in children aged 5 years and older in 2018. The availability of the ASIT drug in children is expected to be highly significant, given that the number of patients with pollinosis is increasing rapidly, as noted above. More recently, safety and expected immunological responses were demonstrated for combination therapy using a Japanese cedar SLIT tablet with a house dust mite SLIT tablet.⁹⁷ ASIT for multiple sensitizations is also becoming more realistic. However, Japanese cedar pollen SLIT is not a panacea, and approximately 20% of patients would be poor responders.⁹⁸ While it has been suggested that the therapeutic effect of 3 years of SLIT drop medication should persist for 2 years after the discontinuation of dosing, the basis for the decision to discontinue or restart treatment remains unclear.⁹⁹ Exploratory research on both predictive and surrogate biomarkers will be required to resolve these issues.

Therapeutic effect of Japanese cedar pollen ASIT on Japanese cypress pollinosis

Another issue related to Japanese cedar pollen ASIT is its effectiveness against Japanese cypress pollinosis. To address this issue, we first review the current clinical situation. As mentioned above, given the cross-reactivity that exists between the major pollen allergens of Japanese cedar and Japanese cypress, ASIT using extracted Japanese cedar pollen is expected to act on Th2 responses, not only against Japanese cedar allergen-specific Th2, but also Japanese cypress allergen-specific Th2. Indeed, Japanese cedar pollen allergen-specific SCIT or SLIT can inhibit both Cry j 1-specific IL-5 production and Cha o 1-specific IL-5 production.^{11,55,100} However, the effectiveness of Japanese cedar pollen SCIT in treating patients with Japanese cedar/cypress pollinosis has been reported to be reduced during the Japanese cypress pollen season.^{11,101,102} In another study, a survey was performed on 180 patients with Japanese cedar/cypress pollinosis who received Japanese cedar pollen SLIT. Surprisingly, 54.9% of these patients reported that their allergic symptoms worsened during Japanese cypress pollen season.¹⁰³ In addition, among 37 good responders during Japanese cedar dispersal season, TNSMS worsened (by more than 1 point) in 17 patients (45.9%).¹⁰³ Thus, unfortunately, Japanese cedar pollen ASIT is not necessarily effective against Japanese cypress pollinosis.

Below we present four hypotheses that may explain why Japanese cedar pollen ASIT is only partially effective against Japanese cypress pollinosis.

1. Presence of T cell epitopes specific to major allergens in Japanese cypress pollen

It is possible that, although Japanese cedar pollen ASIT reduces the response of cross-reactive (shared T cell epitope-specific) T cells, T cells specific to Japanese cypress do not respond to Cry j 1

exposure. This treatment would therefore only induce partial immune tolerance to Cha o 1 (Fig. 2A).

2. Presence of B cell epitopes specific to major allergens in Japanese cypress pollen

Cry j 1 treatment (with Japanese cedar pollen ASIT) cannot induce all variations of Cha o 1-neutralizing IgGs, such as IgG4 antibodies, if Cha o 1 and Cry j 1 possess distinct B cell epitopes (Fig. 2B).

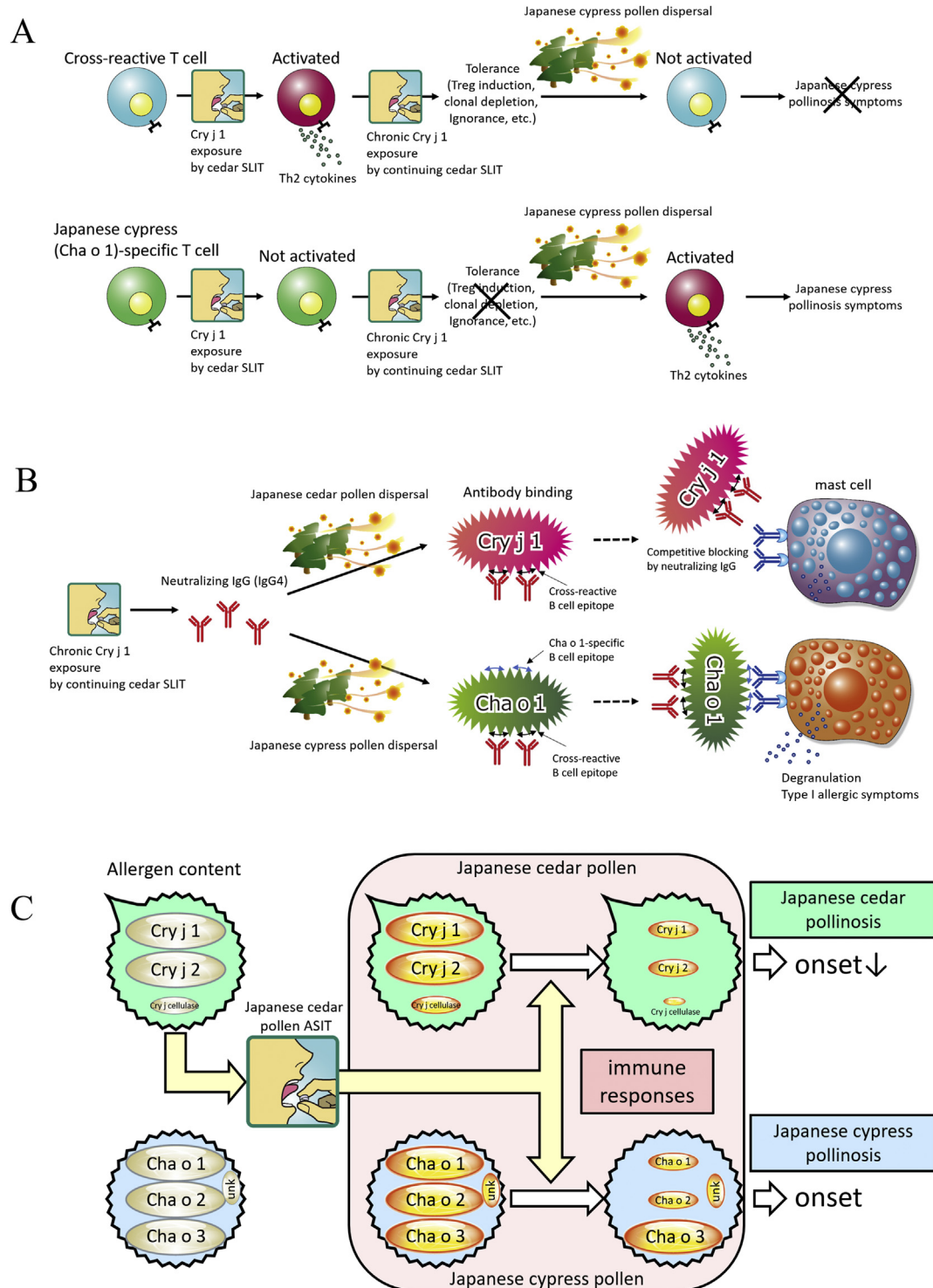


Fig. 2. Four hypotheses explaining why Japanese cedar pollen ASIT is not effective against Japanese cypress pollinosis. **(A)** Hypothesis 1: Presence of T cell epitopes specific to major allergens in Japanese cypress pollen. Such epitopes have already been determined with Cha o 1, 2, and 3. **(B)** Hypothesis 2: Presence of B cell epitopes specific to major allergens in Japanese cypress pollen. Few studies have investigated Japanese cypress pollen allergen-specific antibodies. **(C)** Hypotheses 3 and 4: Presence of a dominant or unique (respectively) allergen in Japanese cypress pollen. Regarding Hypothesis 3, Cha o 3 is currently considered to be the dominant cypress allergen. Japanese cedar pollen ASIT (SCIT or SLIT) can suppress Cry j 1, Cry j 2 and Cry j cellulase-specific immune (Th2) responses. ASIT also inhibits Cha o 1- and Cha o 2-specific Th2 responses on cross-reactive T cells. Recent reports suggest that Cha o 3-specific Th2 responses will remain following Japanese cedar pollen ASIT, given the low content of Cry j cellulase in Japanese cedar pollen. Since there are multiple unidentified allergens in Japanese cypress pollen, we consider that the Cha o 3-Cry j cellulase case may be just the tip of the iceberg. ASIT, allergen-specific immunotherapy; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; unk, unknown allergens unique to Japanese cypress pollen.

3. Presence of a dominant Japanese cypress pollen allergen (Cha o 3)

Treatment for over 2 years by Japanese cedar pollen SCIT did not completely control Cha o 3-specific IL-5 production, despite the fact that Cry j 1-, Cry j 2-, and Cha o 1-associated Th2 cytokine production was completely suppressed.⁵⁵ As mentioned above, the amount of Cry j cellulase in standardized cedar pollen extract for SCIT/SLIT may be insufficient for controlling the Cha o 3-specific Th2 response (Fig. 2C).³⁰ Similarly, it has been reported that Japanese cedar pollen SCIT does not reduce Japanese cypress extract-stimulated induction of Th2 cytokines.^{11,104}

4. Presence of allergens unique to Japanese cypress pollen

In a competitive inhibition test, the addition of Japanese cedar pollen extracts to patient serum samples did not completely inhibit binding to Japanese cypress pollen antigen.¹⁰⁵ Although this result may reflect the presence of Cha o 3 in Japanese cypress pollen (Hypothesis 3), the existence of other unique and unknown allergens in cypress pollen cannot be excluded (Fig. 2C).

Most recently, Kikuoka *et al.* reported novel findings addressing this question. Surprisingly, administration of Japanese cedar pollen extract SLIT over a period of 1–2 years inhibited both Cha o 1- and Cha o 3-specific IL-5 production, whereas SLIT did not induce Cha o 1- or Cha o 3-specific production of IL-10 and Cha o 1- or Cha o 3-specific IgG4; in contrast, this treatment did induce Cry j 1-specific production of IL-10 and IgG4.¹⁰⁰ A similar study showed an increase in Japanese cedar-specific IgG4, but not in Japanese cypress-specific IgG4, among patients treated with Japanese cedar pollen SCIT for more than 3 years.¹⁰⁶ It might be necessary to harmonize not only Th2 cytokine suppression, but also the production of allergen-specific inhibitory cytokines (such as IL-10) by inducing Treg and IgG4, an allergen-neutralizing antibody, in order to suppress allergic symptoms. These findings suggest that, while Japanese cedar pollen ASIT can modulate immune responses against Japanese cedar pollen allergens leading to immune tolerance, this treatment cannot induce substantial Japanese cypress pollen allergen-specific immune tolerance, including that to Cha o 3. Unfortunately, Japanese cypress pollen ASIT is not feasible at this time, given that no standardized Japanese cypress pollen extract is available in Japan. ASIT using Cha o 3 or Japanese cypress pollen extract, or more preferably, a combination treatment comprising both Japanese cedar and Japanese cypress pollen extracts, would be required to optimally control allergy symptoms during the spring Cupressaceae pollen season.

Conclusions

Japanese cedar and Japanese cypress pollinosis are considered to be closely related, if not identical, based on the cross-reactivity of their major allergens. Indeed, it has been proposed that Japanese cypress pollen inhalation with cross-reactive allergens merely re-challenges Japanese cedar-specific immune responses, implying that there is no such disease as Japanese cypress pollinosis. However, recent advances in Japanese cedar pollen ASIT, Japanese cypress pollen allergen research, and the unique situation in which two types of Cupressaceae pollen are released sequentially in Japan have revealed that while Japanese cedar pollinosis and Japanese cypress pollinosis are clearly similar, but *not* the same disease. Acquired immune responses against Taxodiaceae-subfamily (cedar) allergens differ from those against Cupressaceae-subfamily (cypress) allergens, despite the sequence homologies that exist between allergens from the two subfamilies. We propose that Japanese cypress pollinosis is caused by a sensitization mechanism that is independent of that caused by Japanese cedar pollen. We further propose that, unfortunately, the established Th2 response against the major allergens of these two tree species can cross-react

with one another in some patients. We hope that this article will provide an opportunity to review the pathogenesis and pathophysiology of Japanese cedar and cypress pollinosis, thereby facilitating advances in research on diagnostic tests and novel ASIT against both Japanese cedar and Japanese cypress pollinosis.

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Conflict of interest

TO is a salaried employee of Taiho Pharmaceutical. MO received lecture fees from Taiho Pharmaceutical, Mitsubishi Tanabe Pharma, Novartis, and Sanofi.

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