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Dynamics of airborne pollen particles from inhalation to allergic reaction in the nose*

M. Okuda[1,2], K. Ohkubo[2], M. Gotoh[2], K. Hiroshima[1], Y. Ishida[1], K. Hori[1]

[1] Japan Allergy & Asthma Clinic, Tokyo, Japan
[2] Department of Otolaryngology, Nippon Medical School, Tokyo, Japan

SUMMARY

***Background**: Pollinosis is common worldwide, and has been frequently studied. However, the intranasal dynamics of pollen grains have not yet been documented. The purpose of this study is to elucidate for the first time the dynamics of Japanese cedar pollen (JCP) in the human nose at consecutive steps from inhalation to allergic reaction together with release of Cry j 1 (a major allergenic component of JCP) in the nose.*

***Methods**: A personal sampler collected airborne pollens at head height outdoor on the street, while intranasal pollens after natural or experimental inhalation were collected by irrigation with 200ml saline. Cry j 1 in the supernatant after in vitro incubation with phosphate buffered saline or lavage was determined by enzyme-linked immunoassay.*

***Results**: Head-height pollen was 183.0 ± 43.1/300L/h, with 99% of the inhaled pollens deposited on the nasal surface. Eighty eight% of the inhaled pollen was transported to the out-side of the nose by ciliary function within 3 hours. During this process, considerable amounts of Cry j 1 were released in the nose reaching its plateau within 30 min. When the number of pollen deposited exceeded more than approximately 65 particles, symptoms may occur, leading presumably up to a 74% reduction of the intra-nasal pollen.*

***Conclusion**: The majority of inhaled airborne pollens was deposited on the nasal mucosal surface and moved out from the nose by mucociliary transportation. During this process, when allergenic substances are released up to a critical concentration, allergic reactions occur leading to expelling of pollen from the nose followed by subsiding of the symptoms.*

Key words: Japanese cedar pollinosis, aerobiology, intranasal pollen, Cry j 1

INTRODUCTION

Pollinosis is a common disease worldwide. Although not life-threatening, its impacts on the quality of life, health problems and productivity in the workplace are significant. A large number of studies have therefore been targeted at the allergological, ecological and botanical aspects of pollinosis.

The acute nasal symptoms of pollinosis are generally thought to be the result of several consecutive steps: inhalation of pollen into the nose, deposition of pollen particles onto the nasal surface, elution of allergenic substances from the particles and their binding with specific IgE antibodies on the nasal surface mast cells, release of chemical mediators by the degranulation of these mast cells, leading to stimulation of target tissues by these mediators. However, to the authors' knowledge, no studies have yet documented in detail the human intranasal dynamics of airborne pollen from inhalation to symptom manifestation, because it is difficult to count allergen particles in the nose.

Approximately 13% of the population of Japan suffers from Japanese cedar pollinosis (JC pollinosis) [1,2]. This disease, which is specific to Japan, has therefore been extensively studied, and provides a good model for the study of the intranasal dynamics of airborne pollen. The major allergen in Japanese cedar pollen (JCP) is Cry j 1[3]. The purpose of this study is to elucidate for the first time the dynamics in each of the consecutive steps, leading to symptom manifestation. This paper presents the results of a series of in vivo experiments including the measurement of the pollen count in air at head height, evaluation of the changes in pollen count in the nose after inhalation and deposition, and quantification of the release of Cry j 1.

MATERIAL AND METHODS

Subjects

All subjects underwent allergy tests including skin testing, an assay for specific IgE antibodies in the serum and a test for

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nasal eosinophilia. The diagnosis of pollinosis was made based on these results. The six authors of this paper (3 males and 3 females, age range 39 to 76 years), who all had negative test results, acted as examiners and as subjects to be examined in this study. In addition, 18 patients with Japanese cedar pollinosis (8 males and 10 females, the mean age of 39.2 ± 13.4 years) and 13 normal controls (5 males and 8 females, the mean age of 36.4 ± 10.1 years) who had visited our clinic for diagnosis or treatment, and were naturally exposed by a usual number of airborne pollen. These patients with pollinosis were symptomatic but had not had any medicament treatment at least one week prior to visit. These two groups participated in the irrigation protocol to investigate the intranasal pollen count to natural pollen exposure as described below. Informed consent was obtained from all subjects. The local institutional review board approved this study from the ethical point of view.

Airborne pollen count

Airborne JCP were collected with a Durham's collector [4] on a rooftop of five pollen survey stations (20m above ground level) continuously in central districts of Tokyo during this study period (from 3 - 12 March, 2001). Pollen grains were stained with Carberla's solution on a Vaseline-coated glass slide, and the numbers were counted using a light microscope. The mean number of pollen grain per 1 cm^2 of the slide was calculated. The mean daily pollen count was 100.5 ± 41.2 per 24 hours.

During this study period, airborne JCP were also collected by a portable personal volumetric sampler. Each subject wore the sampler for 1 hour or more at head height (approximately 1.5 meter above the ground) on a total of 23 streets in Bunkyo-ku, an area of central Tokyo. The sampler used consisted of a cylinder type inlet tube with a cross sectional area of 3.14cm^2 (156% of that of the average adult nostril) and length of 4cm, connected to a glass fiber filter (25mm in diameter, 0.2mm thick, model GA-55, Toyo Roshi, Tokyo, Japan). The pores of the glass fiber filter were small enough to trap all grains of JCP (average diameter of 30micron) that entered into the tip. The sampler was run at a constant flow of 5L/min (the average minute ventilation of Japanese adults). Grains of JCP collected on the filter were stained with Calberla's solution and counted using a light microscope.

Experimental pollen inhalation

A pollen inhalation device consisted of a pollen generator (20ml flask), pollen exposure chamber (200cm^3 box), nozzles or a facemask and a compressor. One small spoonful (3mm diameter, 1mm deep hollow) JCP (about 170,000 grains) was placed into the generator. A positive pressure of 0.5kg/cm^2 was then introduced to send the pollen into the exposure chamber. Adjusting this pressure could control the amount of pollen in the exposure chamber.

Intranasal pollen count

We investigated the recovery of whole pollen grains from the nose by nasal washing. Five subjects without pollinosis inhaled a known quantity (about 10,000 grains) of JCP from the pollen inhalation device. Then intranasal JCP was collected by bilateral nasal irrigation with 200ml of warm saline solution using Okonogi's nasal irrigation instrument [5] (Figure 1). During irrigation we asked the subjects for continuous phonation of the syllable "A" in order to close the nasopharyngeal space so as to avoid leaking of irrigation solution from the nose to the pharynx. This irrigation was repeated on alternating sides for a few minutes (see legend of Figure 1). The saline solution thus collected was measured (mean quantity of recovered saline solution was 194.37 ± 7.93ml in 16 subjects), and then vacuum filter through a glass fiber filter. JCP on the filter was stained and counted.

We repeated this washing procedure three times consecutively in the same subjects without further pollen inhalation to investigate how many repetitions are necessary for the recovery of all intranasal pollen grains. Individual nasal lavage was separately collected and the number of pollen grains was quantified.

We investigated pollen grains that were deposited in the nose and expired from the nose in experimental pollen inhalation. Subjects without pollinosis inhaled pollen through the nozzles of a pollen chamber. At the end of the inspiration phase the nozzles were quickly removed from the nostrils, and air was expired through the nostrils into a vinyl bag. This procedure was repeated at a natural rate of breathing for 10 seconds. Pollen in the bag were washed out, filtered and counted; this result was taken as the number of pollen grains in the expired air. Nasal pollen was collected by the irrigation method described above and this result was the number of grains of pollen deposited in the nose.

The percentage mucociliarly clearance (MCC) of pollen grains on the mucosal surface was also measured. In a pilot study we insufflated carbon powder particles in the nose and measured times necessary for complete disappearance of these particles in subjects with non-allergic or with allergic rhinitis. The mean clearance times were 15.1 ± 8.1 min in 42 subjects with normal nose and 14.8 ± 10.4 min in subjects with allergic rhinitis, showing no significant difference. Next, immediately after (i.e., at baseline), and 30min and 180min after experimental pollen inhalation with the inhalation device, intranasal JCP was separately collected and counted as described above. The percentage MCC was calculated as the pollen count at time 30 or 180 min divided by the count at baseline, multiplied by 100.

We also investigated the effect of nasal allergic reaction on reducing intranasal pollen counts. We measured the difference in the mean intranasal pollen count between the 18 pollinosis and 13 non-allergic subjects at the time of their visit to our clinic during the study period. Their demographic characters and irrigation method have already been described above.

Figure 1. Okonogi's irrigation instrument. With this instrument, a saline solution is introduced through nozzles into one nostril and collected from the other; during this irrigation, subjects close the nasopharyngeal space by elevating the soft palate through the phonation of the syllable "a". This irrigation was repeated on alternating sides for a few minutes. A volume of 194.37 ml ± 7.93 ml of 200 ml (n=16) was recovered.

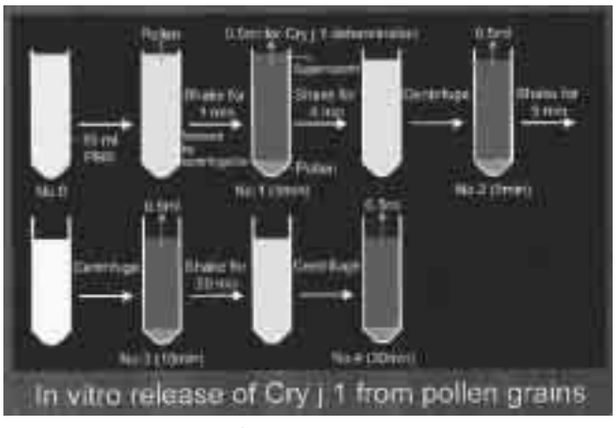

Figure 2. Determination of in vitro Cry j 1 release. First, 0.5ml PBS without pollen was taken as baseline concentration (No.0). For the second determination, either 2,430 or 38,280 JCP grains were incubated in 10 ml PBS in a test tube, and gently shaken once every 1 sec for 1 min. The solution was then centrifuged at 1,000 rpm for 10 sec, and 0.5 ml of the supernatant was taken for determination of Cry j 1 content (No.1). The remaining pollen in the PBS solution were then shaken again for a further 4 min, centrifuged, and another aliquot of the supernatant was removed for Cry j 1 determination (No.2). This procedure was repeated twice more, after shaking for 5 (No.3), and 20 min (No.4). The remaining volumes of pollen-PBS solution after each iteration were therefore 10, 9.5, 9.0 and 8.5 ml. With each iteration, the number of pollen grains remained constant but the volume of the solution decreased. We corrected this bias by calculating the Cry j 1 concentration as the measured Cry j 1 concentration per ml multiplied by 10, divided by the volume of the solution.

Determination of Cry j 1

We examined the release of Cry j 1 from JCP in vitro and in vivo. In the in vitro experiment, JCP grains were incubated in 10ml phosphate buffered saline solution (PBS, M/15, pH 7.2) in a polyethylene test tube, and after shaking and centrifugation, 0.5ml of the supernatant was taken for determination of Cry j 1 content. This procedure was repeated at 1, 4, 5 and 20min for determination of Cry j 1 released during the incubation periods of 1, 5, 10 and 30 min (Figure 2).

The in vivo release of Cry j 1 was also measured. Five non-allergic subjects inhaled JCP using the inhalation device. Ten minutes later, nasal washing with 200ml saline solution was performed. The resulting saline solution was vacuum filtered; 0.5ml of this filtrate was used in the determination of Cry j 1 content.

Determination of Cry j 1 was made in duplicate by means of sandwich enzyme immunoassay [6]. We used anti-Cry j 1 antibody (LCD Allergy Institute, Osaka, Japan) on the immunoplate as primary antibody, biotin-conjugated anti-Cry j 1 antibody as secondary antibody, streptavidin-galactosidase as reactive enzyme and o-nitrophenyl-beta-D-galactopyranoside as substrate.

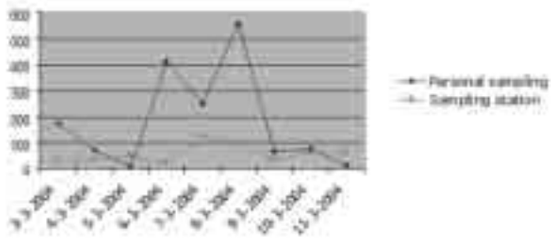

Figure 3. Daily variation of airborne pollens during the study period. The square line indicates pollen counts collected per one hour by a personal sampler on various streets of Bunkyo-ku, an area in central Tokyo, and the diamond line indicates the mean pollen counts collected by a Duhram's collector for 24 hours at 5 survey stations in central districts in Tokyo. The study period from 3 to 12 March 2001 was the middle of the pollen season.

RESULTS

The period between 3 and 12 March 2001 was the middle of the airborne JCP season according to the rooftop pollen count results and the daily report by the Tokyo Pollen Survey Station as shown in Figure 3. Airborne JCP at head height was 183.0 ± 4 3.1/300L air/h (n=23).

Since the sampler filtered at a rate of 5L/min, the average minute ventilation of Japanese adults, the average adult in central Tokyo, could be expected to be exposed 183.0 ± 43.1 JCP grains per hour.

In the five non-allergic subjects, the recovery of pollen was 90.0 ± 4.48% after the first saline wash, 6.46 ± 2.69 after the second and 2.85 ± 1.60% after the third. We therefore concluded that the first nasal irrigation with 200ml saline solution recovered almost all of the JCP in the nasal cavity (Figure 4). Recovery of saline solution after irrigation was also satisfactory as described in the Material and Methods chapter.

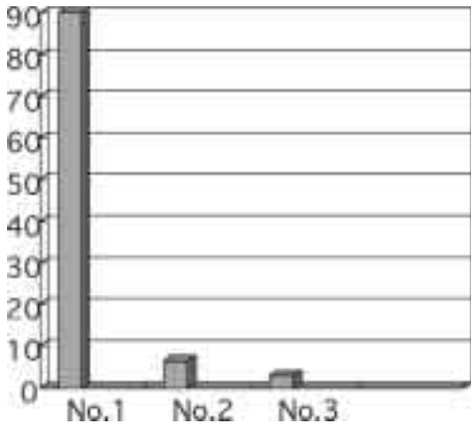

Figure 4. Recovery of intranasal pollen grains by irrigation. One min after inhalation of pollen grains (about 10.000) from the inhalation device, the nasal cavity was irrigated with 200ml PBS. This procedure was repeated three times and each lavage fluid was separately collected, filtrated and then the pollen grains were counted. The majority of intranasal grains was washed out in the first irrigation. The result indicates that a single irrigation with 200ml of PBS can collect almost all pollen grains from the nose.

We also investigated the differences in intranasal deposition and mucociliarly clearance of inhaled pollen in non-allergic subjects. In experimental inhalation, regardless of the number of pollen inhaled (780,810 and 17,278 grains), almost all pollen were deposited in the nose. However, when a small number of grains were deposited in the nose, these grains were cleared more quickly than when extreme larger number of grains were deposited (Table 1).

The number of intranasal pollen grains was different for the 13 non-symptomatic non-allergic subjects (65.00 ± 85.73 grains per patient) versus 18 symptomatic patients with cedar pollinosis (16.53 ± 23.30 grains per patient). This suggests that an allergic reaction reduces the amount of pollen in the nose (74.6% reduction).

The in vitro release of Cry j 1 reached a plateau concentration within 30 min incubation. The concentrations per one pollen particle in 10 min incubation with 2,430 and 38,280 were 7.8 pg /ml and 11.5 pg/ml respectively (Figure 5).

Table 1. Mucociliary clearance of intranasal pollen. Intranasal pollen grains were counted by the irrigation method at each timepoint (0, 30 and 180 min) after inhalation of pollen through the inhalation device. About 80% of the pollen grains were cleared within 30 min in No.1 and No. 2 when a small number of grains was initially deposited.

Subjects	Remained 0 min	deposited 30 min	pollen 180 min
No.1	78	11(14.1%)	18(23.0%)
No.2	81	18(22.2%)	19(23.4%)
No.3	17,278	9,344(54.0%)	3,891(22.5%)

% is pollen number at 30 or 180 min per base line(0) after inhalation.

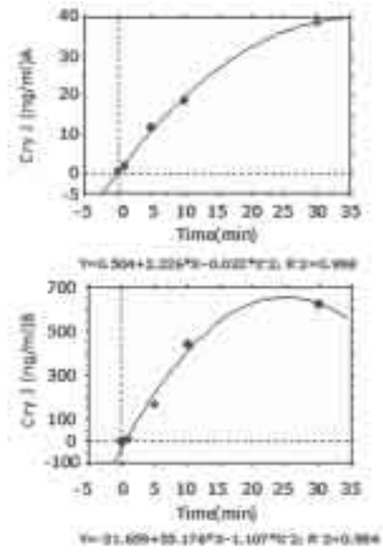

Figure 5. Time course of in vitro release of Cry j1. In determination of Cry j 1 as shown in Figure 2, Cry j 1 from 2,430 JCP grains (A) and 38,230 grains (B) reached a plateau concentration after 30 min of incubation.

Before inhalation of JCP pollen, the average Cry j 1 content in the lavage was 0.31 ± 0.22 ng/ml (62.0 ± 36.14ng total in the 200ml of lavage fluid). Ten minutes after the subjects had experimentally inhaled approximately 8,000 pollen particles (8,047 ± 4,655, n=5), Cry j 1 content in the lavage fluid was 0.76 ± 0.55 ng/ml (152.40 ± 110.30ng in total 200ml of lavage). When the baseline value from Cry j 1 content after nasal pollen inhalation was subtracted, it is estimated that roughly 11.3 pg of Cry j 1 is released from each pollen grain in vivo within ten minutes of its deposition.

Table 2. Dynamics of airborne JCP grains at consecutive stages from inhalation to symptom manifestation. Inhaled pollens were mostly deposited on the nasal mucosal surface and about 80% of it was transported by mucociliarly function to the outside of the nose. During this process Cry j 1 was released from the pollen and reached a critical concentration, leading to symptom manifestation. The symptoms thus induced caused reduction of deposited pollen.

Stage	Pollen count
Inhalation	183
Expiration	2
Deposition*	181
Cleared from nose at 30min**	144.8
Remainging in the nose at 3h	31.7± 58.3
Accumulation before symptom***	67.1 ±16.2
Remainig after allergic reaction****	22.6 ±45.1

The mean pollen count outdoors:183.0 43.0/3000L/h
*inhalation minus expiration
**deposition at 3h
***intranasal pollen in non-allergic subjects
****intranasal pollen in pollinosis patients

DISCUSSION

Based on the results of this study, we can summarize the intranasal dynamics of inhaled JCP as follows. In central Tokyo during the middle of the JCP season, patients would inhale roughly 183 JCP grains per hour, and nearly all of these are deposited on the nasal mucosa. Within 30 min, about 80% of these grains will be transported toward the pharynx. During this time, the JCP releases the antigen Cry j 1 in vivo; from each grain about 11 pg is released within 10 min of its deposition. When the Cry j 1 concentration reaches a threshold, nasal symptoms occur. Up to approximately 74.6% of the pollen on the nasal mucosa may be then expelled through sneezing and watery secretion (Table 2). In this way, the Cry j 1 concentration (pollen number) is again reduced to below threshold. If further deposition of JCP rises above the threshold again, symptoms will recur. This may be one of the reasons why patients have an asymptomatic phase after each allergic attack.

However, there were several problems to overcome in this study. The airborne pollen count varies according to season, time of day, and location. People tend to move to different locations during the day, and typically spend a large part of the day indoors where there is little pollen. Individual exposure to pollen thus depends highly on habits and time of year. Individual sensitivity to pollen antigens also varies. Accordingly, the number of pollen particles inhaled in this study may be different under different experimental conditions and from person to person.

This study was designed with combinations of in vivo and in vitro, natural and experimental pollen exposure, and subjects with pollinosis and those with normal nose.

We conducted studies on pollen deposition, mucociliarly clearance, and in vivo elution of Cry j 1 in subjects without pollinosis but not in subjects with pollinosis. Because allergic patients were challenged with pollen, reactions induced could reduce the number of intranasal pollens. However, the results of this study may be supported by the following evidences; intranasal pollen deposition was examined as short as 10 seconds after inhalation. This time is too short to induce allergic reaction even in pollinosis patients; mucociliarly function was not different in subjects between pollinosis and non-pollinosis subjects in the carbon particle clearance test; Cry j 1 released per one pollen particle in vitro was comparable with that in vivo, suggesting that Cry j 1 released in the nasal surface was collected before its escape in the tissue; it has been reported that roughly 100 grass or ragweed pollen grains can induce symptoms [7,8]. Our previous data [9] show that nasal challenge with 65.0 ± 79.6 grains of JCP can also induce nasal itching within 5 to 10 min. This pollen count was comparable with intranasal pollen (65.0 ± 85.7) in subjects without pollinosis, who visited us on natural pollen exposure during the study period. Thus, the difference in intranasal pollen count between subjects with and without pollinosis suggested that pollen grains were expelled from the nose by an allergic reaction. However, further study is needed to confirm this result in patients with pollinosis whose reaction to pollen had been inhibited by pre-treatment with anti-histamine or topical anesthesia.

In conclusion, we found that in patients with JCP pollinosis in the middle of the pollen season the number of intranasal pollen grains changed at each step of pollinosis. The majority of pollen inhaled is deposited on the nasal mucosal surface and 80% of that is transported by mucociliarly function toward the pharynx within 30 min, if an extreme large number of pollen is not inhaled. During this process Cry j 1 is released and reaches a plateau within 30 minutes leading to symptom manifestation. After the manifestation intranasal pollen grains reduce below a critical concentration. This is the first report to elucidate systemically the dynamics of pollen grains in the human nose, counting the number of pollen particles at each step of the process: inhalation, deposition, transportation after deposition, accumulation. There is a critical pollen count before symptom manifestation and a decrease immediately after the symptom manifestation.

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M. Okuda
2-15-2, Mukogaoka, Bunkyo-ku,
Tokyo, 113-0023
Japan

Fax: +81-35685-6286
E-mail: mok655nms.ac.jp@nifty.com