

Carica papaya pollen allergy

Carlos Blanco, PhD; Nancy Ortega, MD; Rodolfo Castillo, MD; María Alvarez, MD; Antonio G Dumpierrez, MD; and Teresa Carrillo, PhD

Background: *Carica papaya* (CP) trees are widely cultivated in tropical and subtropical areas; however, CP pollen allergy has not been previously described.

Objective: To study patients with CP pollen hypersensitivity.

Methods: A CP pollen extract was elaborated. Skin prick tests (SPTs) with this extract, as well as with commercial papaya fruit and papain extracts, were performed. Specific IgE levels to CP pollen, papaya fruit, and papain were determined. Specific conjunctival challenge tests to the CP pollen extract were also performed. RAST inhibition studies among CP pollen, papaya fruit, and papain were carried out. Twenty atopic patients were used as a control group for in vivo and in vitro tests.

Results: Six patients with clinical histories of seasonal rhinoconjunctivitis or bronchial asthma in relation to CP trees exposure, suggestive of IgE-mediated respiratory allergy, were studied. Commercial SPT and specific serum IgE to papaya fruit and papain were positive in our patients. An IgE-mediated hypersensitivity to a CP pollen extract was demonstrated in all patients, by means of SPTs, specific serum IgE determinations, and conjunctival challenge tests. Control atopic subjects showed negative SPTs, specific IgE, and conjunctival challenge tests to the CP pollen extract. On RAST inhibition studies using CP pollen extract in solid phase, a significant crossreactivity was found among CP pollen, papaya fruit, and papain.

Conclusions: Our study suggests that papaya flower pollen is able to induce respiratory IgE-mediated allergy. The existence of common allergens among papaya flower pollen, papaya fruit, and papain has been demonstrated by RAST inhibition.

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INTRODUCTION

Carica papaya (CP) is a tree belonging to the Caricaceae family, native to Central America. It is widely distributed throughout the tropical and subtropical areas of the world, including the Canary Islands, and is usually cultivated in small plantations or home gardens. *Carica papaya* pollination is basically carried out by wind and aided by small insects. It flowers and produces fruit throughout the year although pollination in our area occurs mainly during the spring and summer months. Its edible fruit, the papaya, is a favorite breakfast and dessert fruit which can be used to make drinks,

salads, marmalade, and candies. Furthermore, papain is a protease enzyme with a molecular weight of 23 kD, obtained from the latex of the papaya fruit, which has many industrial applications. Papain is used widely as a meat tenderizer, clearing agent in the production of beer, contact lens cleaner, and as a reagent in the biochemical and pharmaceutical industries.¹

IgE-mediated reactions induced by papaya and papain ingestion have been described,²⁻⁵ and occupational IgE-mediated asthma has been induced by inhalation of papain.⁶⁻⁹ Further, chymopapain is obtained from papain. Chymopapain is used in intradiscal injection for the treatment of patients with herniated intervertebral discs, a procedure called chemonucleolysis, that induces IgE-mediated anaphylaxis in approximately 1% of the patients treated.^{4,10-14}

We studied six patients with rhinitis and/or bronchial asthma related to CP tree exposure during CP pollination months. We have demonstrated, both by in vivo and in vitro methods, that an IgE-mediated hypersensitivity to CP pollen was responsible for these symptoms.

PATIENTS AND METHODS

Patients

Patient 1. A 40-year-old woman, housewife, with a 10-year history of perennial rhinoconjunctivitis and bronchial asthma, showing house dust mite and seafood hypersensitivities, was admitted to our service. For the previous 3 years she had noticed respiratory symptoms worsening from spring to autumn months that she had claimed was due to CP cultivation in a garden next to her house. She had never eaten papaya fruit.

Patient 2. A 9-year-old boy with a 3-year history of rhinoconjunctivitis from spring to autumn time when exposed to CP trees on his father's farm and also while playing with cats. He had eaten papaya fruits without problems.

Patient 3. A 16-year-old man, student, with a 1-year history of severe rhinoconjunctivitis during the weekends from spring to autumn months when visiting his parents who lived near CP plantations. He lived in a city, where he had been suffering mild perennial naso-ocular symptoms for the last 6 years. He tolerated the ingestion of papaya fruit.

Patient 4. A 14-year-old boy, student, with a 4-year history of mild perennial rhinoconjunctivitis who showed house dust mite and pollen hypersensitivities. During the last 2 years, he had noticed severe rhinoconjunctivitis during the spring and summer months when exposed to CP trees

From the Section of Allergy, Hospital Universitario Ntra. Sra. del Pino, Las Palmas de Gran Canaria, Canary Islands, Spain.

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that had been cultivated in his village. He had never eaten papaya fruit.

Patient 5. A 21-year-old man, student, with a past history of pollen-induced rhinoconjunctivitis and asthma plus food hypersensitivity, manifested an oral allergy syndrome to nuts and mustard. He had noticed a 2-year worsening of the respiratory symptoms that he had claimed was related to CP cultivation near his house. He had never eaten papaya fruit.

Patient 6. A 22-year-old man, electrician, with a 4-year history of rhinitis during the summer months, showed hypersensitivities to dust mite, cat, and grass pollen. He had noticed worsening of the rhinitis symptoms when exposed to CP trees cultivated next to his workplace. He tolerated papaya fruit ingestion.

Pollen Extract

Carica papaya pollen was collected from male flowers during autumn months. The pollen was examined microscopically to exclude contamination by other pollens or fungi. The extract was prepared by incubation of pollen in phosphate buffered saline (PBS) pH 8 at 15% (wt/vol) for 4 hours, at 4°C with magnetic stirring, followed by 30 minutes, 4°C, centrifugation at 10 000 *g*. The supernatant was dialyzed against PBS for 24 hours at 4°C, sterilized by passage through a 0.22- μ m filter (Millipore, USA), frozen at -40°C, and freeze dried. Protein content, assessed by the Bradford method (BIORAD, USA), was 3%.¹⁵ Extracts for in vivo and in vitro studies were prepared by dissolving the lyophilized in PBS at 10% (wt/vol) in sterile vials, and then stored at 4°C until used.

Papaya Fruit Extract

A papaya fruit extract was elaborated by extracting proteins at 10% (wt/vol) in PBS pH 7.4 during 4 hours, at 4°C, with magnetic stirring, and centrifuging at 10 000 *g* for 30 minutes. The supernatant was then dialyzed against PBS for 24 hours at 4°C, sterilized by passage through a 0.22- μ m filter (Millipore, USA), frozen at -40°C, and freeze dried. Protein content, assessed

by the Bradford method (BIORAD, USA), was 1%. Extracts for in vivo and in vitro studies were prepared by dissolving the lyophilized in PBS at 10% (wt/vol) in sterile vials, and then stored at 4°C until used.

Skin Prick Tests

Skin prick tests (SPTs) were carried out with the CP pollen and papaya fruit extracts made in our laboratory, and with the following commercial aeroallergen extracts: *D. pteronyssinus*, *D. farinae*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *Aspergillus* sp, *Alternaria tenuis*, *Cladosporium herbarium*, *Olea europaea*, *Lolium perenne*, *Artemisia vulgaris*, *Parietaria judaica*, *Blatella germanica*, dog dander, cat dander and papain (CBF-Leti S.A., Barcelona, Spain). Skin prick tests were performed by puncture with a Morrow-Brown needle (Bayer S.A., Madrid, Spain) on the volar side of the forearms. Histamine phosphate (10 mg/mL) was used as a positive control and physiologic saline solution as a negative control. The results were expressed as the mean wheal diameter (in millimeters) after 15 minutes of puncture. A result greater than 3 mm, compared with the saline control, was considered a positive response. Ten pollen-allergic patients sensitized to *Artemisia* and ten patients allergic to dust mites were used as control groups. The same in vivo and in vitro tests were performed in patients and controls. Written informed consent was necessary to participate in the study. The protocol was approved by the investigation and ethics committees of the hospital.

Total and Specific Serum IgE

Levels of total and specific IgE to papaya fruit and papain were assessed by the Pharmacia CAP system (Pharmacia Diagnostics, Uppsala, Sweden), according to the manufacturer's instructions. Specific IgE levels to CP pollen were measured as follows: paper disks were activated with CNBr and coupled with the CP pollen extract as described by Ceska et al.¹⁶ RAST was performed by using the Phadezym RAST technique (Pharmacia Diagnostics, Uppsala, Sweden) according to the manufacturer's recommendations. Levels higher than 0.35 kU/L for CAP and 0.35 PRU/mL for RAST were considered positive results.

RAST Inhibition

RAST inhibitions were done in a pool of sera from our CP pollen-allergic patients, following the protocol of Gleich et al,¹⁷ with some modifications. Briefly, 100- μ L aliquots of the sera were incubated for 1 hour at room temperature with 100 μ L of 10-fold dilutions (1 mg/mL to 100 ng/mL) in PBS containing 0.03% (wt/vol) human albumin, of CP pollen and papaya fruit extracts, and of a papain commercial extract (CBF-Leti, Barcelona, Spain). Phadezym RAST was then performed in duplicate on CNBr-fixed CP pollen disks according to Pharmacia's directions for use. The degree of inhibition was measured in percentage, the zero level being defined as the uptake to the solid phase when the allergen was replaced with PBS. We used an *Artemisia vulgaris* and a *D. pteronyssinus* commercial extracts (CBF-Leti SA,

Table 1. Clinical Features of the Patients*

Patient No.	Age (yr)/Sex	Symptoms	Papaya Tolerance	Associated Sensitivities
1	40/F	RC-BA	?	Mite/Seafood
2	9/M	RC	Yes	Cat
3	16/M	RC	Yes	Mite/Mugwort
4	14/M	RC	?	Pollen
5	21/M	RC-BA	?	Mugwort
6	22/M	R	Yes	Mite/Cat

* F, female; M, male; RC, rhinoconjunctivitis; BA, bronchial asthma; and R, rhinitis.

Table 2. In Vivo Test Results

Patient No.	CP Pollen SPT*	Papaya Fruit SPT*	Papain SPT*	CP Pollen CCT†
1	9	3	15	+
2	10	10	0	+
3	7	0	0	+
4	9	0	0	+
5	7	0	3	+
6	5	0	0	+

* Skin prick tests responses measured as mean wheal diameter (in millimetres).

† Conjunctival challenge test results.

Barcelona, Spain) as negative inhibition controls.

Conjunctival Challenge Tests (CCTs)

Specific CCTs were done with CP pollen extract, following the procedure proposed by Möller.¹⁸ First, SPTs were performed with 10-fold dilutions of CP pollen extract in PBS, starting the CCT with the dilution previous to the first inducing a positive SPT. One drop of the corresponding allergen dilution was then applied on the conjunctiva of one eye, and one drop of PBS on the other eye. The response was evaluated in a blinded manner within 20 minutes of application, and a local reaction of itching, redness, and swelling was considered to be a positive response. If there was no response, the next allergen dilution was then applied until a maximum concentration of 10 mg/mL was reached.

RESULTS

The clinical features of our six patients are summarized in Table 1. Mean age was 20.3 years (range 9 to 40). Three of them had no reaction to papaya fruit

ingestion, and the other three did not regularly consume this fruit. None remembered any adverse reaction to papaya fruit ingestion. One of them, patient No. 6, had been treated with mite immunotherapy for 2 years.

Skin prick tests responses to CP pollen extract were positive in all our patients, to papaya fruit in two patients, and to papain in two patients (Table 2). Skin prick tests responses to CP pollen extract were negative in control subjects. In the same way, CP pollen CCTs were positive in our six patients and negative in control subjects (Table 2).

In vitro test results are shown in Table 3. Mean total IgE was 736 kU/L (range 233 to 1.560). Levels of specific IgE to CP pollen were positive in our six patients and negative in control subjects. Specific IgE to papaya fruit and to papain were also positive in our six patients and negative in controls.

RAST with the pool of sera from our patients against disks marked with CP pollen extract was positive (7.8 PRU/mL). On incubating the pool of sera with the different inhibitor extracts at

increasing concentrations, a progressive RAST-inhibition was obtained, reaching 100% inhibition with the CP pollen extract at the maximum concentration, 72% inhibition with the papaya fruit extract, 99% inhibition with the papain extract, and 50% inhibition with the *Artemisia* extract (Fig 1). On incubating with the *D. pteronyssinus* extract, inhibition was not higher than 20%.

DISCUSSION

We report six patients with clinical histories of seasonal rhinoconjunctivitis or bronchial asthma, suggestive of IgE-mediated respiratory allergy, in relation to CP tree exposure during the CP pollination months. We have demonstrated, by both in vivo and in vitro methods, that a CP pollen IgE-mediated hypersensitivity was responsible for our patient symptoms.

Carica papaya trees are cultivated extensively in tropical and subtropical areas. To the best of our knowledge, however, there are no previous reports on CP pollen allergy. Epidemiologic studies should be carried out to determine the prevalence of CP pollen hypersensitivity in CP growing areas. From another point of view, our study is an example of the importance of knowing the pollens that are present in a geographic area in order to identify new potential aeroallergens.

RAST inhibition results, which showed crossreactivity among CP pollen, papaya fruit and papain, suggesting the existence of common epitopes among them, are also remarkable. Nevertheless, the possibility of a papain RAST inhibition due to enzymatic activity, and not to a real crossreactivity, cannot be excluded. The partial crossreactivity that we have found between CP pollen and *Artemisia* pollen seems to have no clinical implications since both SPTs and CCTs with CP pollen were negative in *Artemisia*-allergic controls. This crossreactivity could be explained on the basis of the possible existence of profilins in CP pollen.¹⁹

Three out of our six patients showed good tolerance to papaya fruit inges-

Table 3. In Vitro Test Results

Patient No.	Total IgE*	CP Pollen Specific IgE†	Papaya Fruit Specific IgE*	Papain Specific IgE*
1	468	14.2	21.9	>100
2	1560	4.2	2.1	8.6
3	233	3.2	5.4	0.5
4	861	8.0	8.1	5.1
5	710	4.7	1.2	0.4
6	585	5.1	2.3	0.7

Levels higher than 0.35 kU/L for CAP and 0.35 PRU/mL for RAST were considered positive results.

* Total and specific IgE in kU/L as measured by CAP.

† Specific IgE in PRU/mL as measured by RAST.

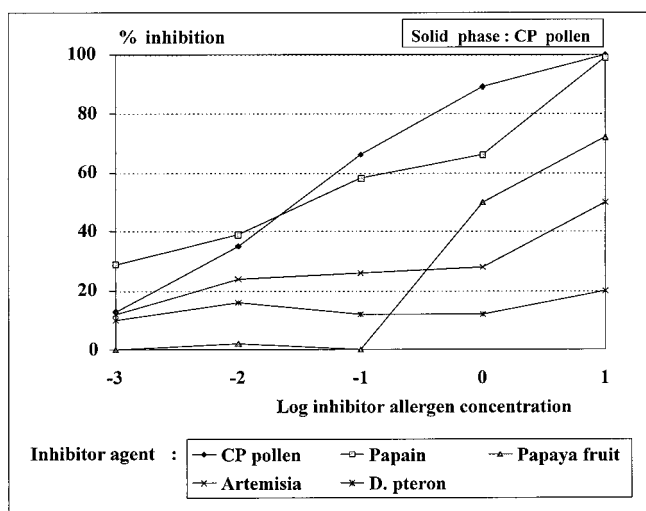


Figure 1. *Carica papaya* (CP) pollen RAST inhibition.

tion, despite positive SPTs and specific serum IgE levels to papaya fruit. The other three patients did not regularly consume this fruit and denied consent to undergo oral challenge tests. It is well known the existence of pollen fruit-associated hypersensitivities,²⁰ and in these patients symptoms of pollinosis usually precede clinically significant food allergy.^{21,22} In consequence, our patients may develop papaya fruit allergy in the future, and a clinical follow up is necessary to rule out this possibility.

Tropical fruit allergy is the most frequent IgE-mediated food hypersensitivity diagnosed among adult patients in the outpatient service of our allergy section, probably due to a high consumption rate of these fruits in our geographic area.^{5,23} It might be interesting to know whether our patients have become sensitized through CP pollen exposure or through papaya fruit ingestion. The presence of clinical symptoms of pollinosis together with good tolerance to papaya fruit suggest the inhalation of airborne allergens as the sensitization route.

None of our patients had formerly undergone chemonucleolysis nor had possible papain contact. Consequently, the most obvious explanation for the papain hypersensitivity we demonstrated in our patients is airborne CP

pollen exposure. One could postulate that the aeroallergen implicated in CP pollinosis could be papain, which is well known to induce IgE-mediated respiratory symptoms.⁶⁻⁹ This hypothesis is supported by the crossreactivity demonstrated between CP pollen and papain although studies are necessary to confirm this fact and to identify other possible CP pollen allergens.

Recently, the increase of CP tree cultivation, due both to the high consumption of papaya fruit and to the industrial applications of papain, suggests that CP pollen allergy could be an important pollinosis in certain areas. Furthermore, the clinical significance of the crossreactivity among CP pollen, papaya fruit, and papain should be investigated further.

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Request for reprints should be addressed to:
Dr Carlos Blanco
Sección de Alergia
Hospital Ntra Sra del Pino
c/Angel Guimerá, 93
35005 Las Palmas de GC
Spain