

RAMIE (*BOEHMERIA NIVEA*) POLLEN-INDUCED BRONCHIAL ASTHMA AND
ALLERGENIC CROSS-REACTIVITY OF RAMIE AND *PARIETARIA*

Naoki Miura

Second Department of Internal Medicine, Nagasaki University School of Medicine

Ramie (*Boehmeria nivea*), a plant of the Urticaceae family, is widely distributed in the Nagasaki area, and has been established to be a cause of asthma¹⁾. The rate of positive reactions to ramie in intradermal tests was 11.7% among adult asthmatic patients²⁾ in the Nagasaki area.

In this study, 10 patients were positive in provocation tests using ramie pollen. Ramie pollen-specific IgE antibodies were measured by ELISA, with the positive provocation test group showing higher O.D. values than the positive intradermal test group ($p < 0.05$).

Ramie is of the same family as *Parietaria*, an important allergen in Europe. The cross-reactivity of ramie and *Parietaria* was examined by an ELISA inhibition test using *P. officinalis* and *P. judaica* (Pj10), but no cross-reactivity was found, suggesting that ramie may be a new independent allergen.

As ramie is widely distributed throughout Japan and South-east Asia, further study is needed to determine whether it is an important allergen of the Urticaceae family in this region, as is *Parietaria* in Europe, and *Urtica* in America.

Key words: allergenic cross-reactivity — *Boehmeria nivea* — ELISA inhibition — *Parietaria judaica* — *Parietaria officinalis*

INTRODUCTION

Allergic conjunctivitis and allergic rhinitis are widely known as allergic reactions to pollen sensitization. In contrast, there are few cases reported of bronchial asthma caused by pollen sensitization other than ragweed in the United States and *Parietaria officinalis* in Europe. Ramie (*Boehmeria nivea*) pollen, which was reported by Asai¹⁾, to be an asthma-causing antigen, produced positive results in 11.7% of intradermal tests²⁾ given to adult bronchial asthma patients. Cross allergenicity between pollen antigens is known to occur within the composite family³⁾, within the rice family⁴⁾, and between *Toxodiaceae* and *Cupressaceae* pollen⁵⁾. Ramie is a member of the

Urticaceae family, and thus belongs to the same family as *Parietaria officinalis*, but to a different genus. A knowledge of cross allergenicity is important for an understanding of antigenic specificity.

This study analyzes examples of ramie pollen asthma in Nagasaki and looks at cross allergenicity with the main European pollen antigens, *P. officinalis* and *P. judaica* (Pj10).

SUBJECTS AND METHOD

1) Subjects

Serum samples from 6 bronchial asthma patients, who were either hospitalized in the Second Department of Internal Medicine, Nagasaki University, School of Medicine or came to the department on a regular out-patient basis, who were confirmed to have ramie pollen asthma in inhalation provocation tests, and who agreed to supply serum samples, were used in antigen cross-reaction tests. Tests were also conducted on serum samples from 20 adult bronchial asthma patients who were positive for ramie in intradermal tests

Received: September 29, 1992

Accepted: January 7, 1993

Abbreviations: BSA bovine serum albumin; LAR late asthmatic response; PBS phosphate buffer saline; PBS-T phosphate buffer saline contained 0.05% Tween 20; PNP p-nitrophenyl phosphate; *P. judaica* *Parietaria judaica*; *P. officinalis* *Parietaria officinalis*

and from 25 patients who were negative.

2) Method

a) Antigens

I) Production of ramie pollen antigen: Ramie pollen was extracted by the dextrose-phenol method to produce an antigen extract. The total protein amount (T.P.) of this extract was 39.4 $\mu\text{g}/\text{ml}$. The extract was lyophilized.

II) *Parietaria officinalis* pollen antigen: *P. officinalis* pollen, purchased from Hollister-Stier, was extracted by the destrose-phenol method to produce an antigen extract. The T.P. was 280.6 $\mu\text{g}/\text{ml}$.

III) *Parietaria judaica* pollen antigen: A major allergen of *P. judaica* is a 10000 dalton polypeptide. This antigen was purchased from Neoabello. The T.P. was 40 $\mu\text{g}/\text{ml}$.

b) Intradermal test

Intradermal test was carried out at 1:1,000 wt/vol dilution of ramie pollen antigen (T.P. 3.9 $\mu\text{g}/\text{ml}$) and determination was based on Ishizaki's method⁶⁾.

The diagnostic allergen intradermal extracts (Torii & Co., Ltd.) were used for the 46 standard antigens.

c) Inhalation provocation test

Based on the standard method⁷⁾ defined by the Inhalation Provocation Test Standardization Committee in the Committee of Bronchial Hypersensitivity, a threshold value was established which determined that a patient was positive in the inhalation provocation test if the forced expiratory volume in one second after inhalation of the antigen fluid was 20% or more below the standard value.

d) Measurement of specific IgE antibody by ELISA method (Fig. 1)

Coating was achieved by adding 200 μl (T.P. 1.4 $\mu\text{g}/\text{ml}$) of ramie pollen antigen diluted with a pH 9.6 sodium carbonate buffer to each well of an ELISA 96-hole polystyrene microplate (Nunc) and letting it sit overnight at 4°C to encourage adhesion of the antigen to the well.

After this, 1% bovine serum albumin (BSA) (Katayama Chemical Industries) was added and blocking was performed at room temperature for 2 hours. Then, cleaning was carried out 3 times using a PBS containing 0.05% Tween 20 (PBS-T).

Coating : aqueous extract of *B. nivea*
sodium carbonate buffer, pH 9.6 (T.P. 1.4 $\mu\text{g}/\text{ml}$)
overnight at 4°C

Blocking : 1% BSA-PBS
2 h at R.T.

[Washing 3 $\times$]

Patient Serum : 4 $\times$ diluted with
10% BSA-PBS-Tween buffer
5 h at 37°C incubation

[Washing 3 $\times$]

Anti-human-IgE (goat)
alkaline phosphatase conjugate
1000 $\times$ diluted with 1% BSA-PBS-Tween
3 h at 37°C incubation

[Washing 3 $\times$]

Substrate : PNP 1mg/ml diluted with diethanolamine
pH 9.8
1 h at 37°C incubation

NaOH (1N, 100 μl)

Absorbance read at 410 nm using ELISA analyzer

Fig. 1. Procedure of ELISA.

Coating : aqueous extract of *B. nivea*

Blocking

Patient serum 4 $\times$ diluted

addition of : *B. nivea* (T.P. 39.4 $\mu\text{g}/\text{ml} \times 10^{-1} \sim 10^{-4}$)
or
: *P. officinalis* (T.P. 280.6 $\mu\text{g}/\text{ml} \times 10^{-1} \sim 10^{-4}$)
or
: *P. judaica* (Pj10) (T.P. 40 $\mu\text{g}/\text{ml} \times 10^{-1} \sim 10^{-4}$)

3 h at 37°C

Anti-human IgE

Substrate

NaOH

Absorbance read at 410nm

Fig. 2. Procedure of ELISA inhibition.

After adding 200 μ l of the patient's serum, 4-times diluted with a PBS-T containing 10% BSA, and incubating the mixture for 5 hours, cleaning was repeated 3 times with a PBS-T.

Next, as the secondary antibody, alkaline phosphatase conjugated goat antihuman IgE antibody (Tago), 1,000-times diluted with a PBS-T, containing 1% BSA was added, incubation was performed for 3 hours at 37°C in a thermostat, and cleaning was repeated 3 times in a PBS-T.

As the base substance, 100 μ l of PNP (p-nitrophenyl-phosphate) (Sigma) diluted to 1 mg/ml with a 10% pH 9.8 diethanolamine solution was added, incubation was performed for 1 hour at 37°C in a thermostat, 100 μ l of 1 N sodium hydroxide was added to stop the reaction, and an absorbance of 410 nm was measured.

e) *Absorption inhibition test by the ELISA method (Fig. 2)*

Coating and blocking were performed in the

Table 1 Cases of *B. nivea* pollen asthma

Case	Age	Sex	Onset	Season	Positive intradermal test	Intradermal test by <i>B. nivea</i>	Threshold value skin test	P-K reaction	Threshold value provocation test	IgE
R. S.	42	F	39	Autumn	<i>B. nivea</i> HD <i>Candida</i> Ragweed 12 others	$\frac{35 \times 30}{56 \times 55}$ I.P.S.	10^{-7}	+	10^{-4}	590
S. Y.	15	M	3	Autumn Spring Winter	<i>B. nivea</i> HD Cat hair Buckwheat	$\frac{27 \times 17}{58 \times 57}$ I.P.S.	10^{-7}	+	10^{-5}	1480
F. Z.	9	F	2	Autumn Spring	<i>B. nivea</i> <i>Candida</i> 7 others	$\frac{21 \times 15}{40 \times 40}$ I.P.S.	10^{-7}	+	10^{-4}	3700
H. I.	24	F	23	Autumn Winter	<i>B. nivea</i> HD <i>Candida</i> 3 others	$\frac{23 \times 13}{52 \times 42}$	10^{-5}	+	10	880
H. S.	13	M	11	Autumn Rainy Season	<i>B. nivea</i> <i>Humulus</i> HD 12 others	$\frac{11 \times 11}{38 \times 33}$	10^{-4}	+	10^{-3}	1240
K. K.	15	M	10	Autumn Winter	<i>B. nivea</i> HD <i>Candida</i> <i>Alternaria</i>	$\frac{42 \times 27}{62 \times 58}$ I.P.S.	10^{-6}	+	10^{-4}	N.D.
H. M.	12	M	8	Autumn Spring	<i>B. nivea</i> HD	$\frac{35 \times 23}{45 \times 42}$	10^{-6}	+	10^{-3}	462
N. Y.	17	F	4	Autumn Summer	<i>B. nivea</i> HD <i>Alternaria</i>	$\frac{20 \times 15}{25 \times 22}$ I.P.S.	10^{-6}	+	10^{-4}	2850
H. K.	57	M	56	Autumn Winter	<i>B. nivea</i> Ragweed <i>Candida</i> <i>Typha</i> 4 others	$\frac{14 \times 12}{47 \times 27}$	10^{-5}	N.D.	500 $\times$ LAR	270
R. N.	23	M	12	Autumn Spring	<i>B. nivea</i> Ragweed HD <i>Candida</i> 11 others	$\frac{50 \times 18}{75 \times 48}$ I.P.S.	10^{-9}	N.D.	10^{-4}	216

(I=Itching ; PS=Pseudopods ; ND=Not done)

same manner as shown in Fig. 1. Pooled serum from 6 ramie pollen bronchial asthma patients was added to each well together with 100 μ l of either *B. nivea*, or *P. officinalis* or *P. judaica* pollen antigen (Pj10) in a tenfold-dilution with a PBS-T containing 10% BSA. Incubation was performed for 3 hours at a temperature of 37°C in a thermostat, and an absorption reaction took place.

3) Statistical analysis

The ELISA optical density (O.D.) values for the ramie pollen antigen inhalation provocation positive test group, the intradermal positive test group, and the intradermal reaction negative test group were compared using the student's *t* test evaluation.

RESULTS

1) Summary of 10 ramie pollen bronchial asthma patient cases

The age of the patients ranged from 9 to 57, but the average age was a young 22.7 and patients 15 years old or younger accounted for 50% (Table 1). The male-female ratio was almost equal at 6:4. The age at which the illness started was 15 years or younger in 7 cases, and the average age was 16.8 years, indicating that most patients were young. In all 10 cases, the patients were most afflicted in the fall when ramie pollen is scattered in the air. In the intradermal test using 47 standard antigens, 8 of the patients were positive for house dust, and each tested positive for an average of 7.2 antigens. In the intradermal test for ramie pollen antigen, 6 of the patients exhibited strong reactions, including the formation of pseudopods and itching. The threshold value for the group was 10^{-4} or less, and all cases were positive for the P-K reaction. The inhalation provocation threshold value ranged from 10^{-5} to 10^{-1} , and in one case a late asthmatic response (LAR) was observed when using 500-fold diluted antigen. IgE was generally high, with an average of 1298 IU/ml.

2) Comparison of ELISA values

The ELISA values were compared for the ramie pollen antigen intradermal reaction positive and

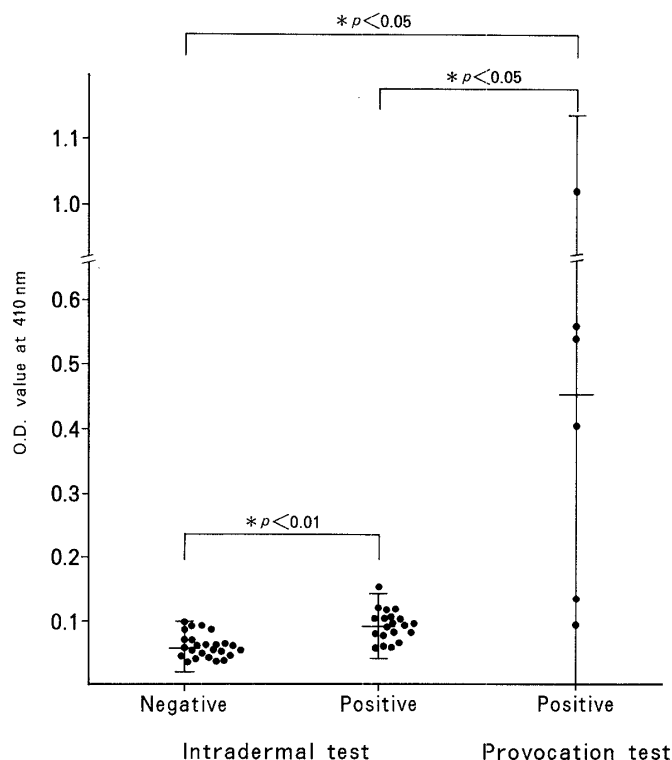


Fig. 3. Relationship between sensitization by *B. nivea* and ELISA O.D. value.

The positive bronchial provocation testing group (AVE $\pm$ 2SD, 0.46 ± 0.68) showed a distinctly higher absorbance ($p < 0.05$) than the positive intradermal testing group (AVE $\pm$ 2SD, 0.094 ± 0.050). The latter group showed a distinctly higher absorbance ($p < 0.01$) than the negative intradermal testing group (AVE $\pm$ 2SD, 0.061 ± 0.039).

negative test groups and inhalation provocation positive test group (Fig. 3). The absorption levels for the intradermal reaction positive group were significantly higher than for the negative test group ($t=5.04$, $p < 0.01$) and it was confirmed that the intradermal positive test group showed an increase in the ramie specific IgE antibody compared to the negative test group.

Also, the inhalation provocation positive group showed significantly higher values than the intradermal reaction positive group ($t=2.64$, $p < 0.05$) and a particularly high amount of IgE antibody was confirmed for this group among all the cases of ramie pollen antigen sensitization. The inhalation provocation positive group also recorded a significantly higher value than the intradermal reaction negative group ($t=2.88$, $p < 0.05$).

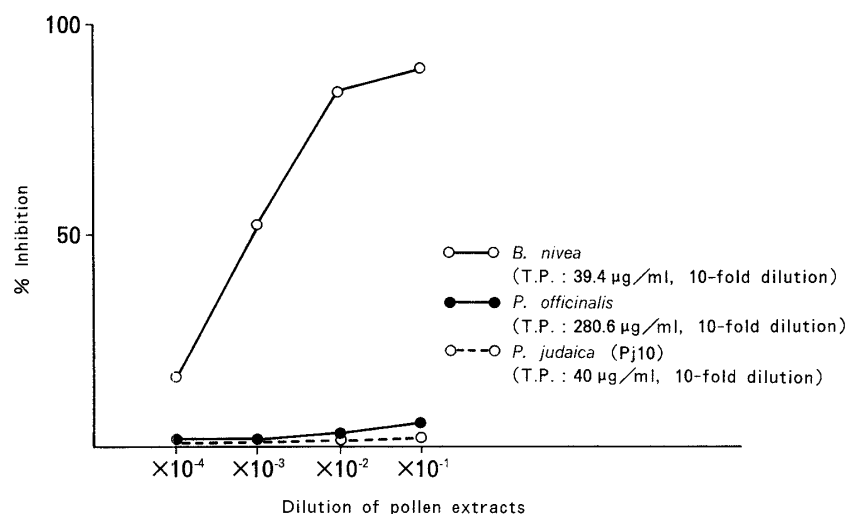


Fig. 4. Inhibition dose-response curve by three pollen extracts.

In a tenfold-diluted series of *B. nivea* inhibition was noted with increase in concentration. For *P. officinalis* and *P. judaica* (Pj 10), however, no allergenic cross-reactivity between *B. nivea*, *P. officinalis*, and *P. judaica* (Pj 10) could be detected in the absence of inhibition.

3) Absorption test using *P. officinalis*, *P. judaica* (Pj 10)

The ramie pollen asthma patients' combined serum was totally absorbed by the ramie pollen antigen (T.P. 39.4 $\mu\text{g}/\text{ml}$) dilution group (Fig. 4). On the other hand, it was not absorbed by the *P. officinalis* (T.P. 280.6 $\mu\text{g}/\text{ml}$) or the *P. judaica* (Pj 10) (T.P. 40 $\mu\text{g}/\text{ml}$) dilution groups. This indicates a lack of allergenic cross-reactivity between ramie and *P. officinalis* or *P. judaica*.

DISCUSSION

Ramie (*Boehmeria nivea*) (Fig. 5-a) belongs to the Urticaceae family and is monoecious, with the female flowers on the upper part of the stem and the male flowers on the lower part⁸⁾. Pollen is dispersed from the stamen of the male flower in large amounts in September. The pollen were 14–16 μm in diameter (Fig. 5-b). The plant grows naturally in clusters on hillsides. It can be found throughout Japan, with the exception of northern Hokkaido, and is distributed widely throughout China and Southeast Asia. Asai¹⁾ first identified the pollen asthma caused by this pollen as ramie pollen asthma, and the present study goes a step further by reporting on 10 cases, indicating that ramie pollen asthma is by no means rare.

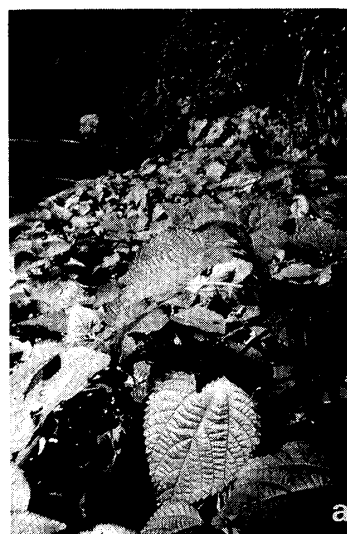


Fig. 5. *Boehmeria nivea*.

a) Ramie (*Boehmeria nivea*) grows in circular clusters on hillsides. b) The pollen were 14–16 μm in diameter.

Since this illness has not been studied in other regions, it is imperative that a history of cases be developed. Common characteristics of the 10 ramie pollen bronchial asthma patient cases (Table 1) included atopic asthma, the young age of patients, peak affliction in the fall, and a high sensitivity to house dust and other standard antigens. Considering the wide distribution of this plant, it is likely that as research progresses,

ramie pollen asthma will be found to be a rather universal pollen asthma.

In measurements of the ramie specific IgE antibody by the ELISA method, the ramie pollen intradermal reaction positive group showed a significantly higher value than the negative group ($p < 0.01$), as did the inhalation provocation positive test group compared to the intradermal reaction positive test group ($p < 0.05$). In most of the inhalation provocation positive test group cases, the ramie pollen specific IgE antibody was found in particularly high amounts, indicating that a strong sensitization is required to trigger asthma.

In the Urticaceae family (Table 2), to which ramie belongs, one also finds the *Parietaria* genus and *Urtica* genus which are important pollen antigens in Europe. *P. officinalis* and *P. judaica* are representative pollen antigens in Western and Southern Europe around the Mediterranean and are known to cause pollinosis and bronchial asthma. In the Barcelona area, 12% of patients showed sensitivity to *P. judaica* (Pj 10) in a prick test, with 27% of these demonstrating bronchial asthma symptoms and 41% pollinosis⁹. In this study, the ELISA inhibition test was used to test for allergenic cross-reactivity between ramie and

P. officinalis or *P. judaica*, but no cross-reactivity was found. However, this test only involved the use of serum from 6 Japanese patients with ramie pollen bronchial asthma. It is necessary to confirm this result by performing such tests on more Japanese cases, and at the same time to conduct tests using serum from Europeans sensitive to *P. officinalis* or *P. judaica*. Also, in these 6 cases, it was confirmed that clearly different antigen determination sites were the source of sensitivity.

Parietaria has not been reported as a cause of allergy in North America¹⁰. Of the *Parietaria* genus, only *Parietaria micrantha* has been confirmed in Japan. It grows naturally in small amounts in the Kanto and Chubu areas. However, there are no reports of allergic illnesses caused by this plant in Japan. In North America, 25% of atopic patients tested positive in skin reaction tests for *Urtica dioica* but the clinical picture was unclear¹¹. In Japan, *Urtica thunbergiana*, *U. platyphylla*, *U. laetevirens*, and *U. angustifolia* are found, but not *U. dioica*, and there are currently no cases of bronchial asthma reported in connection with these. The presence of allergenic cross-reactivity between *Parietaria* and *Urtica dioica* has been largely rejected¹²⁻¹⁴. Research on the cross allergenicity of ramie and *Urtica* is also necessary to confirm the independent features of the ramie pollen within the Urticaceae family.

In Nagasaki, many family homes are located on hillsides, and clusters of ramie can be found growing throughout the area. As a result, the pollen dispersion concentration is very high and high sensitivity is likely. In the Nagasaki area, ramie has proven to be the most important plant among the Urticaceae family causing pollinosis and bronchial asthma.

Since ramie is distributed widely across Eastern and Southern Asia, it is likely that future research will reveal ramie to be the most important pollen antigen of the Urticaceae family in Asia, similar to *Parietaria* in Europe and *Urtica* in North America.

The author thanks Professor Kohei Hara, Dr. Sadahiro Asai, for their guidance, and the members of the Allergy Study Group of the Second Department of Internal Medicine, School of Medicine, Nagasaki University for their assistance.

Table 2 Urticaceae family

<i>Urtica</i>	—	*	<i>U. dioica</i>
			<i>U. thunbergiana</i>
			<i>U. platyphylla</i>
			<i>U. laetevirens</i>
			<i>U. angustifolia</i>
<i>Laportea</i>			
<i>Pilea</i>		*	<i>B. nivea</i>
			<i>B. spicata</i>
<i>Nanocnide</i>			<i>B. tricuspis</i>
			<i>B. formosana</i>
<i>Elatostema</i>			<i>B. sieboldiana</i>
			<i>B. biloba</i>
<i>Pellionia</i>			<i>B. kiussiana</i>
			<i>B. pannosa</i>
<i>Boehmeria</i>			<i>B. holosericea</i>
			<i>B. longispica</i>
<i>Villebrunea</i>			<i>B. platanifolia</i>
<i>Debnegeasia</i>		*	<i>P. officinalis</i>
			<i>P. judaica</i>
<i>Parietaria</i>			<i>P. lusitanica</i>
			<i>P. mauritanica</i>
<i>Pouzolzia</i>			<i>P. micrantha</i>
<i>Gonostegia</i>			* causes allergic reaction

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カラムシ花粉喘息及びヒカゲミズ属との交差反応についての研究

長崎大学医学部第二内科

三浦直樹

カラムシ (*Boehmeria nivea*) は、イラクサ科の植物であり、長崎地方に広く分布し、その花粉は、喘息誘発抗原として確立されている。長崎地方では、成人気管支喘息患者のカラムシに対する皮内反応陽性率は11.7%と高率を示した。

本研究では、吸入誘発試験により確診した、カラムシ花粉気管支喘息患者10例を報告した。カラムシ特異 IgE 抗体を ELISA を用い測定したところ、カラムシ花粉抗原吸入陽性群は皮内反応陽性群よりも有意に高値を示した ($p < 0.05$)。ヨーロッパの重要な花粉抗原である *Parietaria* とは同科異属であり、*P. officinalis* 花粉抗原および *P. judaica* 花粉抗原との共通抗原性を ELISA 抑制試験を用い検討したが、認められず、カラムシ花粉は新しい独立抗原であると考えられた。

カラムシは日本をはじめとして東南アジア一帯に広く分布しているところから、今後研究が進めば、イラクサ科花粉の中で、ヨーロッパにおける *Parietaria* や米国における *Urtica* のような重要な花粉抗原になると考えられた。