

***Taxus cuspidata* (Japanese yew) pollen nasal allergy**

Shiroh Maguchi ^{a*}, Satoshi Fukuda ^b

^a Department of Otolaryngology, Teine Keijinkai Hospital, 1-12 Maeda, Teine-ku, Sapporo 060-8638, Japan

^b Department of Otolaryngology, School of Medicine, Hokkaido University, N15W7, Kita-ku, Sapporo 006-8555, Japan

*Corresponding author. E-mail address: smaguchi@fa2.so-net.ne.jp (S. Maguchi)

Abstract

We have investigated the *Taxus cuspidata* pollinosis that has never been studied before. We applied immunoblotting method in order to detect specific IgE antibody against *T. cuspidata* using its pollen itself. Out of 18 patients with seasonal allergic rhinitis suffering mainly in April and May, 5 patients were sensitized with this pollen. Furthermore, we were able to diagnose one case as *T. cuspidata* pollinosis from the clinical and laboratory findings. While the birch pollen is the main causative pollen in the spring, *T. cuspidata* is one of the minor allergens causing pollinosis in our district.

Keywords: pollinosis, Japanese yew, *Taxus cuspidata*, immunoblotting

1. Introduction

It is well known that the birch (*Betula platyphylla* var. *japonica*) is a representative cause of the tree pollinosis in Hokkaido, the island of northern part of Japan, whereas Japanese cedar (*Cryptomeria japonica*) pollen is a major allergen causative of tree pollinosis in Japan [1]. This is the same as observed in northern Europe [2]. The birch pollen keeps scattering heavily every year in Hokkaido in

spring from late April until early June, and many pollinosis patients see otolaryngologists during this time.

However, the number of nasal allergy patients begins to increase actually from early April every year. As the pollen of birch is not airborne early April, we assumed that the cause of the pollinosis at that time was alder pollen that scatters at the beginning of April and that it had a cross reactivity with birch pollen [1].

In 1998, in order to verify this hypothesis, we surveyed 68 nasal allergy patients whose nasal symptoms began early or in mid April, before the flowering of birch. The result was that the causative pollen was mainly alder pollen as expected, which was followed by elm and poplar pollen. However, approximately 20 % of those patients indicated no sensitization to birch, alder, poplar, elm, or mite allergens [unpublished data].

The airborne pollen grains for flowering in Sapporo in April are those of alders (*Alnus*), yews (*Taxus*), elms (*Ulmus*), poplars (*Populus*) and birches (*Betula*). It is impossible to find the sensitivity against *Taxus* pollen in those five kinds of pollen, because there are neither commercially available tests to examine its specific IgE antibody nor diagnostic extracts from that pollen. Therefore, we assumed the possibility that the patients who began nasal allergy symptoms before flowering of birch and had no sensitivity to alders, elms, poplars or birches were sensitized to *Taxus* pollen.

Then, we introduced a technique of immunoblotting for the detection of *Taxus* pollen-specific IgE using the pollen grain itself, and investigated whether the pollinosis due to *Taxus cuspidata* is present or not in Sapporo.

2. Materials and methods

2.1. *Taxus cuspidata* (Japanese yew)



Fig. 1. *Taxus cuspidata* (Japanese yew) in April. It has many small, solitary flowers.

Taxus cuspidata (Japanese yew) is a very slow-growing and long-lived tree. It reaches a height of 25 m and a diameter of 2 m. It is widely distributed in Japan, Sakhalin, Kuril, Korea and the north-eastern section of China. Although it is widely distributed from the north to the south in Japan, it is abundant in Hokkaido. The leaves are flat, 1.3 - 4 cm long, and pale on the underside. Narrow and sharply pointed, they are arranged spirally around the twig. Small, solitary flowers appear in the spring (Fig. 1). The diameter of its pollen is 20-26µm and has a little square form (Fig. 2).



Fig. 2. The pollen of *Taxus cuspidata* (Japanese yew). (400x)

2.2. Analysis of airborne pollen

The Durham type pollen samplers are situated in Hokkaido University, which is located in the center of Sapporo city. The analysis of pollen samples was carried out microscopically throughout the pollen season in 1998 and 1999. The numbers of each pollen type were counted within 4 cm² on the slides after dying with Carberla solution (glycerin 5 ml, ethanol 10 ml, 2 drops of saturated fuchsine and distilled water 15 ml).

2.3. Measurement of IgE antibody

2.3.1. Patient sera

Serum samples were obtained from 18 patients with seasonal allergic rhinitis living in or around Sapporo City and suffering mainly in April and May every year. The samples were stored at -80°C until use.

2.3.2. Measurement of IgE antibody by immunoblotting

Since the diagnostic extracts for *Taxus cuspidata* are not commercially available, we applied a method to measure its IgE antibody

with immunoblotting technique according to the method of Takahashi et al [3], with some modification.

The pollen of *Taxus cuspidata* were collected directly from flowering plants in April 1998, and kept at -4°C until use. Birch (*Betula platyphylla* var. *japonica*) pollen and alder (*Alnus maximowiczii*) pollen were used for positive control tests, which had been collected in 1991 and stored at -4°C.

Respectively small amounts of the three kinds of pollen (birch, alder and yew) were placed 10 mm apart from each other on a nitrocellulose membrane (5 x 50 mm, Bio-Rad Laboratory, CA, USA), which had been treated with 0.05M Glycine buffer pH 7.6 containing 20% methanol for 1h to transfer the pollen allergens. The membrane was then washed and blocked with TRIS-buffered saline pH 7.8 (TBS) containing 4% bovine serum albumin for 1h at 37°C. The membrane was rinsed three times with TBS containing 0.25% Tween 20 (washing buffer) and treated with the patient's undiluted serum overnight at room temperature. The membrane was then washed with a large volume of washing buffer and treated with biotinylated anti-human IgE (Kirkegaard & Perry Lab., Inc., Gaithersburg, MD, USA) for 1h at 37°C. After vigorous washing with washing buffer, it was treated with biotinylated avidine alkaline phosphatase complex (Vectastain ABC-AP. AK-5000 of Vector Laboratories. Inc. Burlingame, CA, USA) for 30 min at 37°C. Again, the membrane was washed with a large volume of washing buffer, and then treated with BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium), a phosphatase substrate kit (Kirkegaard & Perry Lab., Inc., Gaithersburg, MD, USA) for 30 min at 37°C. The allergens were revealed as purple spots as a result of this procedure. The amount of specific IgE antibody was estimated from the degree of the coloring in three steps, -, +, 2+, respectively.

2.2.3. Measurement of IgE antibody by CAP radioallergosorbent test (CAP RAST)

A CAP RAST (Pharmacia, Uppsala,

Sweden) was performed according to the manufacture's instructions to measure IgE antibodies against the two kinds of pollen, birch and alder.

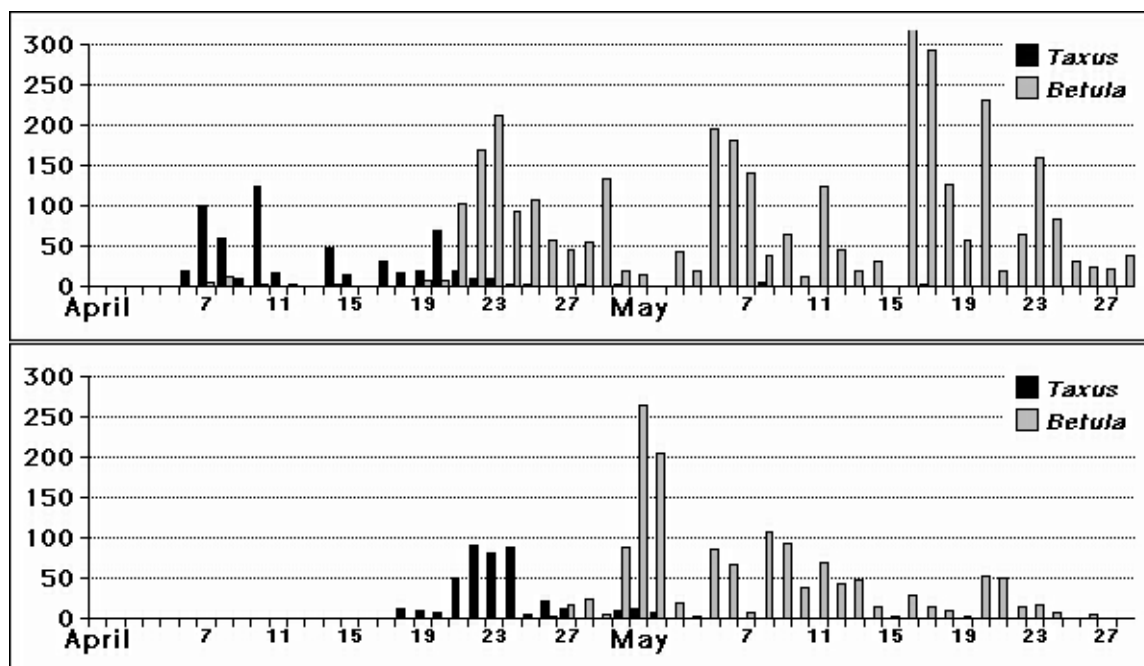


Fig. 3. Airborne pollen counts of *Taxus* and *Betula* in 1998 and 1999.

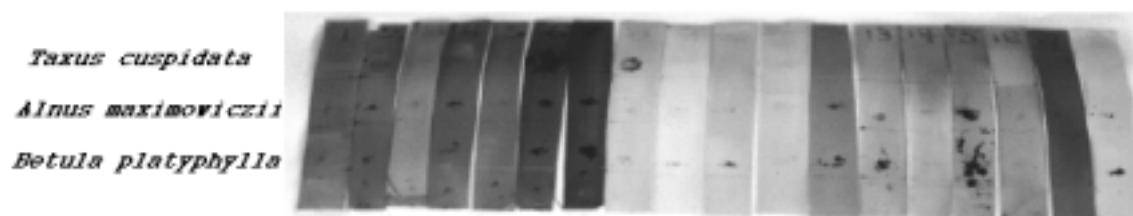


Fig. 4. The membrane with immunoblotting of specific IgE against *T. cuspidata*, *A. maximowiczii* and *B. platyphylla*.

3. Results

3.1. Airborne *Taxus* pollen counts

Airborne *Taxus* pollen counts are shown in Fig. 3. Japanese yew started its flowering early April in 1998 and middle in April in 1999, approximately 2 weeks before the beginning of birch flowering in both years. The total counts of yews were 584 / 4 cm² in 1998 and 405 / 4cm² in 1999, and it is one third to one sixth of the total counts of birch

pollen, 3748 / 4 cm² in 1998 and 1415 / 4 cm² in 1999.

3.2. Specific IgE antibody examined with immunoblotting and CAP RAST

We have examined 18 sera of seasonal nasal allergy patients. The membrane with immunoblotting of specific IgE against *Taxus cuspidata*, *Alnus maximowiczii* and *Betula platyphylla* is shown in Fig. 4. The specific IgE antibody levels expressed by the degrees of

coloring and the titer obtained by CAP-RAST are listed in Table 1. By immunoblotting method, 5 out of 18 sera (27%) were found positive for IgE antibody against *Taxus cuspidata*, 16 out of 18 (89%) sera were positive against *Alnus maximowiczii* and 14 out of 18 sera (78%) were positive against *Betula platyphylla*.

The relationship between IgE antibody against birch by CAP-RAST and the appearance of spots by immunoblotting with *Betula platyphylla* is shown in Fig. 5, where a good correlation was observed. However, there were 2 sera which were negative by CAP-RAST but positive by immunoblotting method (No. 1 and No. 16).

On the other hand, there was no correlation between IgE antibody against birch by CAP-RAST and the appearance of spots by immunoblotting with *Taxus cuspidata* as shown in Fig. 6.

Out of six patients (No. 1, 3, 4, 6, 14, 15) whose nasal allergy symptoms began early or middle in April, before the flowering of birch, only two patients had specific IgE antibody against *Taxus cuspidata*. Furthermore, 3 patients (No. 1, 3, 14) who were negative for birch or alder by CAP-RAST were also negative for *Taxus cuspidata* by immunoblotting, but unexpectedly positive for *Alnus maximowiczii* or *Betula platyphylla* by immunoblotting.

Serum No.	Immunoblotting			CAP RAST(KU/l)	
	<i>Taxus cuspidata</i>	<i>Alnus maximowiczii</i>	<i>Betula platyphylla</i>	Alder	Birch
1	-	+	+	<0.34	<0.34
2	+	+	+	N.D.	13.30
3	-	+	-	<0.34	<0.34
4	+	+	+	7.93	8.46
5	-	-	-	<0.34	<0.34
6	2+	2+	2+	N.D.	33.60
7	-	2+	2+	31.80	54.40
8	2+	+	+	N.D.	4.18
9	-	+	+	N.D.	8.00
10	-	+	+	15.70	24.70
11	+	+	+	N.D.	4.77
12	-	+	+	N.D.	8.44
13	-	+	2+	N.D.	36.30
14	-	+	-	N.D.	<0.34
15	-	+	2+	48.30	78.30
16	-	+	+	N.D.	<0.34
17	-	-	-	N.D.	<0.34
18	-	+	+	6.19	9.05

N.D.=not done

Table 1. The specific IgE antibody levels measured from the degrees of coloring by immunoblotting and the titer obtained by commercially available CAP RAST.

4. A case report

There was a case which we were able to diagnose definitely as *Taxus cuspidata* pollinosis from the clinical symptom, IgE antibody against its pollen, and the result of provocation test.

The patient was 43-year-old female who had been complained of nasal allergy symptoms every year in the spring since 1985. She visited an outpatient clinic with a complaint of serous nasal discharge and nasal congestion on May 28th, 1991. Her nasal symptoms began early April in 1997 and 1998, and middle in April in 1999, before the flowering of birch. Laboratory examination revealed many eosinophils in her nasal discharge and her CAP-RAST scores were: house dust, 0; mite, 0; birch, 3; alder, 3; elm, 0; poplar, 0; orchard, 0; and mugwort, 0. IgE antibody against *Taxus cuspidata* was + by immunoblotting method (No. 4), and nasal provocation test using the grains of the pollen was also positive. From these results, we diagnosed her as having *Taxus cuspidata* pollinosis.

5. Discussion

Although Takahashi et al. reported the presence of IgE antibody against *Taxus* pollen in the sera of nasal allergy patients [2], there have been no report about the definite *Taxus* pollinosis patient.

In our study, we reported a case of *Taxus* pollinosis patient, which presented a specific IgE antibody and the nasal symptoms were easily provoked by *Taxus* pollen. Therefore, we decided that the patient's complaints in early April were due to *Taxus* pollen.

Since the diagnostic extracts of *Taxus cuspidata* pollen are not commercially available, we applied immunoblotting technique in order to detect specific IgE antibody. As the

correlation between IgE antibodies against birch detected by CAP-RAST determination and the appearance of spots by immunoblotting with *Betula platyphylla* showed, this measurement is fully reliable (Fig. 5). In addition, we decided that there was no cross reactivity between *Betula* and *Taxus*, as there was no correlation between IgE antibodies against those two kinds of pollen (Fig. 6).

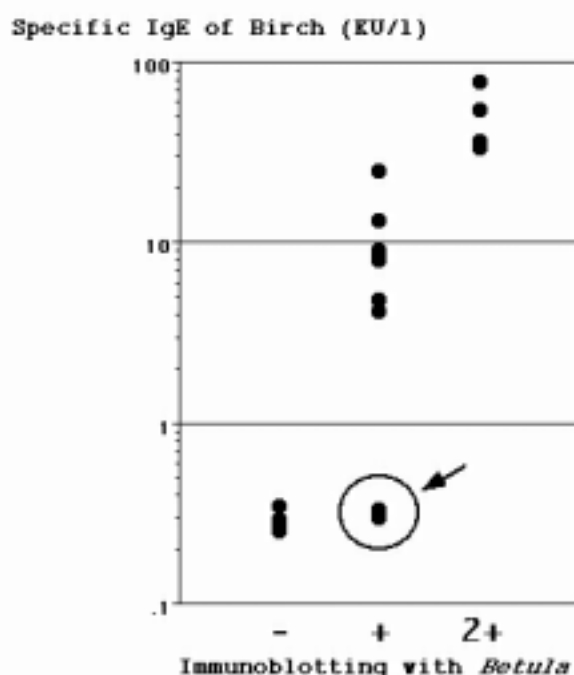


Fig. 5. The relationship between IgE antibody against birch by CAP-RAST and the appearance of spots by immunoblotting with *Betula platyphylla*. The arrow indicates two sera which were negative by CAP-RAST but positive by immunoblotting method.

By this immunoblotting method, 5 out of 18 sera (27%) were positive for IgE antibody against *Taxus cuspidata*. This result indicates that *Taxus cuspidata* is one of the allergens that cause pollinosis in the spring in our district. However, the ratio of 27% is apparently low, compared to the ratio of *Alnus maximowiczii* (89%) or *Betula platyphylla* (78%). Furthermore, all 5 sera, positive for *Taxus cuspidata*, were simultaneously positive for *Alnus maximowiczii* and *Betula platyphylla*. It

indicates to us that we should take *Taxus cuspidata* pollen as a minor allergen to cause pollinosis in our district.

At first, we assumed that the patients who began nasal allergy symptoms before flowering of birch and had no sensitivity detected by commercially available CAP-RAST had *Taxus cuspidata* pollinosis. However, sera of such 3 patients were negative for *Taxus cuspidata* but positive for *Alnus maximowiczii* or *Betula platyphylla* by immunoblotting (Fig.5). It demonstrates that immunoblotting is more sensitive than commercially available CAP-RAST determination in the measurement of IgE antibody. The allergens used were *Betula verrucosa* for CAP-RAST of birch and *Alnus incana* for alder. Both grow in Europe and they are different from the Japanese birch and alder in species, even though they are included in the same genus. We assume that the low sensitivity observed in CAP RAST determinations might be due to the difference.

5. Conclusion

In Hokkaido, the northern island of Japan, the most important causative allergen of pollinosis in spring is birch pollen. However, *Taxus cuspidata* pollen scatters every year before birch flowering.

We investigated whether the *Taxus cuspidata* pollen causes pollinosis in our district by immunoblotting method, since we could not detect specific IgE antibody using commercially available test. In conclusion, we found a definite case of pollinosis due to this pollen. Although *Taxus cuspidata* pollen is not a major pollen causing pollinosis, we should take it as one of minor allergens that causes nasal allergy in early spring in Hokkaido.

Specific IgE of Birch (KU/l)

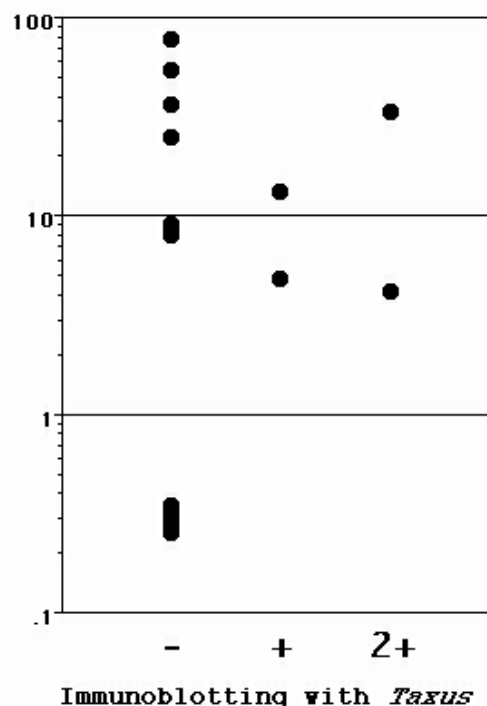


Fig. 6. The relationship between IgE antibody against birch by CAP-RAST and the appearance of spots by immunoblotting with *Taxus cuspidata*.

References

- [1] Maguchi S, Takagi S, Yoshida M, Fukuda S, Inuyama Y. Birch pollen nasal allergy in Sapporo and its cross reactivity with alder pollen. (in Japanese). J Otolaryngol Jpn 1993; 96:1-9.
- [2] D'Amato G, Spiekma FThM, Liccardi G, Jäger S, Russo M, Kontou-Fili K, et al. Pollen-related allergy in Europe. Allergy 1998; 53:567-578.
- [3] Takahashi Y, Nilsson S, Berggren B. Aeroallergen immunoblotting with human IgE antibody. Grana 1995; 34:357-360.