Comparison of Swedish and Japanese Venom Antigens in the Diagnosis of *Hymenoptera* Hypersensitivity in Japan*

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SUMMARY **We compared the usefulness of Swedish and Japanese venom antigens in cases of *Hymenoptera* stings by using intracutaneous tests and radio-allergosorbent tests (RAST) on 15 hypersensitive subjects. The intracutanous tests with both Swedish and Japanese *Hymenoptera* venoms showed positive reactions to paper wasp and yellow-jacket venom antigens in 73 per cent and 93 per cent of the subjects, respectively. The optimal concentration of the venom antigens was 1.0 μg/ml. RAST with antigens of both Swedish and Japanese venoms gave positive responses to wasp and yellow-jacket venom antigens in 60 to 80 per cent of the same group of subjects. None of the normal subjects, none of whom any history of sting hypersensitivity, had a positive response to the intracutaneous tests or RAST. These results suggest that both Swedish and Japanese venom antigens have similar antigenicity and that they are useful in diagnosing *Hymenoptera* hypersensitivity in Japan.**

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Hymenoptera stings not infrequently cause anaphylactic reactions in individuals who have been sensitized to the venom by a previous sting, and deaths due to severe anaphylactic shock are occasionally reported in Japan. Thus, correct diagnosis of *Hymenoptera* hypersensitivity is very important in order to start immunotherapy with corresponding venom antigens so as to prevent potentially fatal anaphylactic reactions. *Hymenoptera* comprises several subfamilies, e.g. *Apidae, Vespidae* and *Formicidae.* The honeybee and bumblebee are the most important *Apidae.* The Polistes wasp, the hornet and the yellow-jacket are the most important species of *Vespidae.* Wasp and yellow-jacket stings have been identified as being the most common cause of allergic reaction.[1,2] Honeybee hypersensitivity is freqently found among occupational beekeepers, but it is not commonplace among the population at large in Japan.[3,4]

The antigenicity of venoms differs among species of *Hymenoptera,* although it has been reported[5-8] that wasp and yellow-jacket venoms are similar in antigenicity while that of the honeybee is different from that of *Vespidae.*[9,10] For the diagnosis of *Hymenoptera* hypersensitivity, although it is ideal to use the venom of the insect species which caused the hypersensitive symptoms, various kinds of diagnostic venom antigens are not available currently. Moreover, it is difficult to identify accurately the species of the causative insect. Recently, several venom antigens have become commercially available for skin tests and radio-allergosorbent tests (RAST). In this study, commercial venom antigens from Sweden are compared with antigens prepared from wasps and yellow-jackets caught in Tochigi, Japan. Using these venoms, intracutaneous tests and RAST were carried out on both normal subjects and *Vespidae*-sensitive subjects. The purpose of this study was to investigate whether or not commercially prepared, imported venom antigens are as useful as domestically prepared antigens for diagnosing *Hymenoptera* hypersensitivity in Japan.

SUBJECTS

The subjects were 11 males and four females with a history of anaphylactic reaction to *Hymenoptera* stings. All of them had experienced generalized anaphylactic symptoms, from mild urticaria and angioedema to more severe reactions such as respiratory difficulty, nausea, vomiting, palpitation, chill, vascular collapse and unconsciousness. Such reactions developed

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within 5-15 minutes after the sting (Table 1).

Fourteen volunteers (six males and eight females) with no history of *Hymenoptera* hypersensitivity served as control subjects. They had no atopic diseases such as atopic dermatitis, allergic rhinitis and bronchial asthma (Table 2).

MATERIALS

Commercial venoms of paper wasps, yellow-jackets and honeybees were obtained from Pharmacia Company, Uppsala, Sweden. Japanese wasp *(Polistes chinensis antenalis Fabricus)* and yellow-jacket *(Vespula flaviceps lewisii Cameron)* venom was prepared from venom-sac extractions. Venom-sacs were collected from the insects which were frozen at -80°C immediately after their capture; 500 milligrams of the sacs were added to 5 millilitres of 50% glycerine-saline solution. They were mixed in a Waring blender for three minutes in ice-cold water and kept overnight at 4°C. The supernatants were collected after centrifugation at 15,000 rpm for 30 minutes; they were clarified by being passed through a millipore (3.0-0.45 μ) membrane filter. The total protein level of these extracts was determined by the Lowry method.

METHODS

Intracutaneous test

A 0.02-ml antigen solution with a concentration of 1.0 μg/ml was injected intracutaneously into each subject's forearm.[11] The diameter of wheal and erythema was measured 15 minutes after the injection. The reactions were graded as follows:

	diameter of wheal		*diameter of erythema*
negative (−)	< 5mm	and/or	⩽9mm
probably + (±)	5 to 8mm	and/or	10 to 19mm
positive (+)	9 to 15mm	and/or	20 to 39mm
strongly + (++)	> 15mm	and/or	⩾40mm

Table 1 Clinical characteristics of *Hymenoptera*-hypersensitive subjects studied

Subj. No.	Sex	Age (years)	Reaction history*	Time lapse# (years)	Serum IgE level (IU/ml)
1	M	69	moderate	1	1066
2	M	43	moderate	1	488
3	F	52	severe	5	133
4	M	54	severe	9	326
5	F	57	moderate	1	187
6	F	51	moderate	1	357
7	M	64	moderate	10	44
8	M	42	moderate	1	463
9	M	57	moderate	1	83
10	M	48	severe	3	145
11	M	40	severe	1	341
12	M	50	severe	19	156
13	F	35	severe	1	349
14	M	31	severe	1	74
15	M	17	severe	1	312

* Moderate symptoms mean anaphylaxis with generalized urticaria or angioedema without loss of consciousness. Severe symptoms mean anaphylaxis with vascular collapse and/or loss of consciousness in addition to generalized urticaria.
#Time lapse between the last sting and diagnostic tests.

Radio-allergosorbent test (RAST)

RAST on Swedish *Hymenoptera* antigens was carried out using commercial RAST kits (Phadebas RAST kit, Pharmacia, Sweden). RAST on Japanese *Hymenoptera* antigens was carried out using Phadebas RAST kits, except for antigen-coupled discs which were prepared by modifying the RAST procedure described by Ceska *et al.*[12] Japanese wasp and yellow-jacket venoms in 20 mg amounts were coupled with 200 CNBr-activated cellulose discs.

A blood sample for each RAST was drawn immediately before the skin test.

RESULTS

Intracutaneous tests

Fourteen out of the 15 hypersensitive subjects showed positive reactions to both the Swedish and the Japanese wasp venoms. Eleven and 12 of them showed positive reactions to Swedish and Japanese yellow-jacket venoms, respectively (Table 3).

None of the 14 normal subjects showed a positive reaction to these antigens. The results suggest that commercial Swedish or Japanese wasp and yellow-jacket venoms at a concentration of 1.0 μg/ml are suitable for diagnosing *Vespidae* hypersensitivity by skin test. At a concentration of 0.1 μg/ml of venom, only four to seven patients showed positive reactions, suggesting that the concentration of antigens was too low to make a correct diagnosis of *Hymenoptera* hypersensitivity. Only two out of the 15 hypersensitive subjects and normal

Table 2 Characteristics of control subjects who had no history of *Hymenoptera* hypersensitivity

Subj. No.	Sex	Age (years)	Serum IgE level (IU/ml)
1	M	31	35
2	F	30	66
3	M	50	NM
4	M	31	106
5	F	31	84
6	F	26	414
7	F	54	167
8	F	25	147
9	M	24	131
10	M	58	321
11	F	31	184
12	F	49	183
13	M	9	432
14	F	34	278

NM= not measured

Table 3 Intracutaneous test with Swedish and Japanese *Hymenoptera* venoms in hypersensitive subjects

Subj. No.	Paper wasp (Pharmacia)	Japanese wasp	Yellow jacket (Parmacia)	Jap. yellow jacket	Honeybee (Pharmacia)
1	+	+	+	+	–
2	+	+	+	+	–
3	+	+	–	±	–
4	+	+	+	+	–
5	+	+	+	+	–
6	±	±	±	±	–
7	+	+	+	+	±
8	+	+	±	+	–
9	+	+	++	+	–
10	++	+	++	++	+
11	++	+	++	++	+
12	++	++	++	++	±
13	+	+	±	+	±
14	+	+	+	+	±
15	+	+	+	±	±
No. of positive reactions	14 (93%)	14 (93%)	11 (73%)	12 (80%)	2 (13%)

subjects showed positive reactions to honeybee venom.

Table 4 shows the relationship of reactions to the intracutaneous test using Swedish and Japanese wasp venoms. Correlation of the strength of an intracutaneous test comparing two antigens was statistically significant at the 5% level using the Chi-square test. All patients except one showed positive intracutaneous test reactions to both the Swedish and Japanese wasp venoms. Table 5 shows the relationship between the reaction of intracutaneous tests using Swedish and Japanese yellow-jacket venoms. The correlation of the strength of an intracutaneous test comparing two antigens was statistically significant at the 5% level using the Chi-square test. Ten out of the 15 subjects showed positive reactions to both the Swedish and Japanese antigens; none of these subjects showed a negative reaction (Tables 4 and 5). None of the control subjects showed a positive reaction to any of the *Vespidae* venoms. These results show that Swedish and Japanese venoms have cross-reacting antigens to skin tests.

RAST

None of the control subjects had a positive RAST score for any of the venoms.

Table 6 shows the results of RAST on each of the hypersensitive subjects. Nine to 12 out of the 15 subjects had a positive score for any one of the venoms from either Swedish or Japanese wasps and yellow-jackets. There was no difference between Swedish and Japanese venoms with regard to the positive rate of RAST. Only two of the subjects had positive RAST scores with honeybee venom. Tables 7 and 8 show the coincidence of RAST with Swedish and Japanese venoms in hypersensitive subjects. Correlations between the RAST scores for Swedish and Japanese venoms (wasp or yellow-jacket) were statistically significant.

Table 4 Relationship of reaction of intracutaneous test with Swedish and Japanese wasp venoms in hypersensitive and normal subjects

Swedish wasp: Skin reactions	Japanese wasp: –	±	+	++	No. of hypersensitive Subj.
–	○○○○○○ ○○○○○○ ○○				0 (0)
±		●			1 (6.7)
+			●●●●●● ●●●●●		11 (73.3)
++			●●	●	3 (20.0)
No. of hypersensitive Subj.	0 (0)	1 (6.7)	13 (86.7)	1 (6.7)	15 (100)

Each open circle stands for a normal subject and each solid circle stands for a hypersensitive subject. The figures in parentheses represent the percentage of total hypersensitive subjects.

Table 5 Relationship of reaction of intracutaneous test with Swedish and Japanese yellow jacket venoms in hypersensitive and normal subjects

Swedish yellow jacket: Skin reactions	Japanese yellow-jacket: –	±	+	++	No. of hypersensitive subj.
	○○○○○ ○○○○○ ○○○○	●			1 (6.7)
±		●	●●		3 (20.0)
+		●	●●●● ●●		7 (46.7)
++			●	●●●	4 (26.7)
No. of hypersensitive Subj.	0 (0)	3 (20.0)	9 (60.0)	3 (20.0)	15 (100)

Notes for this table are the same as those in Table 4.

In RAST with wasp venoms, eight out of the 15 hypersensitive subjects gave positive RAST scores for Swedish and Japanese venoms, while two subjects gave negative RAST scores for both types of venom and five subjects gave positive RAST scores for one of two venoms. In RAST with yellow-jacket venoms, seven out of the 15 hypersensitive subjects gave positive RAST scores both for Swedish and Japanese venoms and three subjects showed a negative RAST score for both venoms, while five subjects gave a positive RAST score for one of two venoms.

DISCUSSION

Among subjects with a history of hypersensitivity to wasp or yellow-jacket stings, both wasp and yellow-jacket venom extracts gave a high percentage of positive reactions in intracutaneous tests and RAST Venoms originating from Swedish and Japanese *Vespidae* were found to be equally potent antigens in these tests. None of the normal subjects studied showed a positive reaction to intracutaneous tests and RAST for any of the venoms.

It is concluded that Swedish commercial venom antigens as well as Japanese antigens can be utilized to diagnose *Hymenoptera* sting hypersensitivity in Japan using intracutaneous tests and RAST as well as venom antigens prepared from Japanese *Vespidae*.

From a biological point of view the *Vespidae* are all in the same family, but separate from the *Apidae* (honeybee). Wasps, yellow-jackets and hornets belong to the *Vespidae* and they have common antigens in the venom. Reisman and others[6] reported that most of the sera from subjects who had experienced allergic reactions after *Vespidae* stings reacted to yellow-jacket and hornet venoms in the RAST analysis. Shulman[13] compared antigenicity of the bee, wasp, hornet and yellow-jacket by using rabbit antisera. A common antigen

was found in the bee and the wasp; it was present in the body, but not in the venom-sac. He found the greatest degree of cross-reactivity between wasp and yellow-jacket venoms. Two common antigens were found, one in the body and one in the venom sac.[13] However, in this study the antigenicity of venoms from Swedish and Japanese *Vespidae* was not exactly identical in RAST. Sera from five out of the 15 hypersensitive subjects showed discordance in RAST with regard to Swedish and Japanese wasp venoms, although in the intracutaneous tests all of these subjects showed positive reactions to both Swedish and Japanese antigens of wasp venom; most of the subjects gave positive reactions to yellow-jacket venom. In fact, Wicher[14] examined the venom immunogenicity of various species of yellow-jacket and found that common sensitivity appears to exist with regard to yellow-jacket venoms except for one species, *Vespula squamosa*. Thus, in the diagnosis of *Hymenoptera* hypersensitivity, it is wise to use more than one venom in skin tests and RAST. In this study, honeybee venom did not give positive reactions in skin tests and RAST in subjects hypersensitive to wasp and yellow-jacket venoms, confirming the previous report that antigenicity of honeybee venom is different from the *Vespidae* venoms. Findlay *et al*,[9] Hoffman[10] and Mitsuhashi[3] reported similar findings by using histamine-release of leukocytes, RAST inhibition test and analysis of antigens.

Skin tests were carried out by using a 1.0 μg/ml concentration of venom antigens. All except one hypersensitive subjects showed positive reactions to all *Vespidae* venoms; none of the normal subjects showed a positive reaction to any of the *Vespidae* and honeybae venoms. In the previous reports,[2,11] we found that by using a 0.1 μg/ml concentration of commercial venom antigens, one third of the patients showed false negative re-

Table 6 RAST with Swedish and Japanese *Hymenoptera* venoms in hypersensitive subjects

Subj. No.	Paper wasp (Pharmacia)	Jap. wasp	Yellow-jacket (Pharmacia)	Jap. yellow jacket	Honeybee (Pharmacia)
1	0	2	0	1	0
2	3	2	2	2	0
3	0	0	0	0	0
4	2	2	2	1	0
5	1	1	1	0	0
6	0	1	1	1	0
7	0	1	0	0	0
8	2	1	2	2	0
9	0	0	2	0	0
10	0	2	2	1	0
11	2	2	3	2	2
12	2	2	0	0	0
13	1	0	1	0	0
14	2	1	3	1	0
15	1	2	3	2	2
Number with >1 scores	9 (60%)	12 (80%)	11 (73%)	9 (60%)	2 (13%)

Table 7 Relationship of RAST score with Swedish and Japanese wasp venoms in hypersensitive and normal subjects

	RAST score	Japanese wasp 0	1	2	3	No. of hypersensitive Subj.
Swedish wasp	0	●● ○○○○○○ ○○○○○○ ○○	●●	●●		6 (40.0)
	1	●	●	●		3 (20.0)
	2		●●	●●●		5 (33.3)
	3			●		1 (6.7)
	No. of hypersensitive subj.	3 (20.0)	5 (33.3)	7 (46.7)	0 (0)	15 (100)

Notes for this table are the same as those in Table 4.

Table 8 Relationship of RAST score with Swedish and Japanese yellow-jacket venoms in hypersensitive and normal subjects.

	RAST score	Japanese yellow-jacket 0	1	2	3	No. of hypersensitive subj.
Swedish yellow jacket	0	●●● ○○○○○○ ○○○○○○ ○○	●●			5 (33.3)
	1	●●	●			3 (20.0)
	2	●	●●	●●		5 (33.3)
	3		●	●		2 (13.3)
	No. of hypersensitive subj.	6 (40.0)	6 (40.0)	3 (20.0)	0 (0)	15 (100)

Notes for this table are the same as those in Table 4

sponse and by using a 10.0 μg/ml concentration, one half of the normal subjects showed a positive, or probably positive, response. These results show that the commercial venom antigens used in this study could be utilized in skin tests for the diagnosis of *Hymenoptera* hypersensitivity. Optimal concentration of venom antigens was 1.0 μg/ml in skin tests. Sixty to 80 per cent of patients showed positive RAST and 70 to 90 per cent of the hypersensitive subjects had positive intracutaneous test results with *Vespidae* venom antigens. All of them showed positive reactions to more than one antigen in RAST and/or intracutaneous tests. Thus, it is advised that both intracutaneous tests and RAST be carried out in subjects who may be hypersensitive to *Hymenoptera* stings.

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