

Comparison of two *in vitro* assays, RAST and CAP, when applied to the diagnosis of anaphylactic reactions to honeybee or yellow jacket venoms

Correlation with history and skin tests

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We compared the results obtained with a new specific IgE assay (Pharmacia CAP system) to those of RAST and intradermal skin tests (ST) performed in 87 patients with a history of generalized reaction to honeybee or yellow jacket venom. When CAP and RAST were compared with positive ST performed with honeybee venom, CAP sensitivity was not significantly higher (98%) than that of RAST (95%). When yellow jacket venom was tested, CAP sensitivity (93%) was clearly superior to that of RAST (40%). When we compared the specificities of RAST and CAP to bee venom, RAST was positive in 21% of the 24 subjects with negative ST, and CAP in 42%. Among the 29 patients with negative ST to yellow jacket venom, RAST was positive in 17% and CAP in 28%. These results do not reflect a lower specificity of CAP, because CAP positivities could be inhibited *in vitro*, and because, in three patients with a history of anaphylactic reaction (one to honeybee, two to yellow jacket), CAP was the only positive test confirming the clinical observation. Among the 53 patients who were able to identify the offending insect (honeybee, 31; yellow jacket, 22), the cause of the anaphylactic reaction was usually confirmed by ST and CAP: honeybee venom 97% for both ST and CAP; yellow jacket venom 82% for ST, 86% for CAP. This was not the case for RAST, which confirmed honeybee venom hypersensitivity in 87% and yellow jacket venom hypersensitivity in only 41%. Thus, CAP is both more sensitive and more rapid than RAST, without losing specificity.

Clinical aspects

An accurate diagnosis of hypersensitivity to Hymenoptera venoms is crucial in patients with a history of anaphylactic reaction, in whom immunotherapy is indicated. Thus, we compared the results obtained with a new specific IgE assay (the CAP system) to those of RAST and intradermal skin tests (ST) for honeybee and yellow jacket venoms. The sensitivity of ST and CAP was shown to be high for both venoms and particularly higher than that of RAST when applied to yellow jacket venom.

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The diagnosis of hypersensitivity to Hymenoptera venoms (honeybee and yellow jacket) is based on a history of anaphylactic reaction, on positive skin tests (ST) to venoms, and on the presence in serum

of venom-specific IgE (4, 11). Since venom immunotherapy is usually performed in patients with a history of a life-threatening reaction, an accurate diagnosis is crucial. ST offer good sensitivity for

venoms of both honeybee and yellow jacket. Serum determination of specific IgE is, however, a useful additional tool, since ST interpretation is sometimes difficult, particularly when ST are performed during the weeks following a sting, when skin sensitivity is reduced by antihistamine or psychotropic drugs and when any skin abnormality is present (10, 12, 16, 20). The results of RAST, a standard *in vitro* method, correlate well with allergic history and ST for honeybee, but not for yellow jacket, venom (17). With the latter, the sensitivity and efficiency of the assay are much lower. For this reason, we considered it to be useful to establish whether CAP, a new, more sensitive test, known to compare favorably with RAST for aeroallergens (7, 14), could also have a better clinical relevance for venoms, particularly for yellow jacket venom.

Material and methods

Subjects

Eighty-seven patients (51 male, 36 female; mean age: 40 years, range 16–65), referred to our allergy unit for investigation of a generalized reaction to honeybee or yellow jacket venom, were selected for the study. In all cases, a detailed history was taken by an allergist and ST, as well as determination of specific IgE, were performed for both venoms (174 results). No subject had taken any drug which could interfere with the ST, and a 6-week delay between insect sting ST and determination of specific IgE was respected. In most cases, the investigations were performed within 6 months after the anaphylactic reaction.

The control group comprised 20 nonatopic volunteers defined as adults not suffering from any past or present disease, with skin prick tests negative for 15 aeroallergens (including mites, mammals, molds, and pollen) and CAP negative for house-dust mite (*Dermatophagoides pteronyssinus*), cat, and pollen of birch and mugwort. In all, total IgE was lower than 80 kU/l.

Skin tests

Venom ST were performed intradermally on the forearm volar surface by the end-point method (end-point titration: 1 µg/ml) with a standardized allergen extract (5) for both honeybee and yellow jacket (Pharmalgen, Pharmacia). The extracts were prepared according to the manufacturer's instructions. A negative (solvent) and a positive (histamine) control were performed. A test was considered to be positive when the diameter of the wheal was 4 mm or greater with a venom concentration of 0.1 µg/ml or less.

Determination of specific IgE in serum

The Phadezym RAST was performed with the two allergen extracts, honeybee and yellow jacket, according to the manufacturer's instructions. Briefly, the allergen, covalently coupled to a paper disk, was incubated with patient serum. After washing, a rabbit anti-IgE, β-galactosidase-labeled antibody was added, and, after a second wash, the optical density was measured. Results were expressed in PRU/ml and graded from class 0 (<0.35 PRU/ml) to class 4 (>17.5 PRU/ml).

CAP was also performed with the two allergen extracts, honeybee and yellow jacket, according to the manufacturer's instructions. Fifty-one determinations were performed in duplicate (honeybee 27 and yellow jacket 24). The technical characteristics have been described elsewhere (3, 6). Briefly, this assay uses a new type of solid phase, that is, a flexible hydrophilic carrier polymer encased in a capsule (CAP), which can bind about three times as much protein allergen as the classical cellulose disk. The test, which can also be performed as a RIA, was used here as a fluorometric assay. The allergen-bound IgE was detected by a mixture of polyclonal and monoclonal enzyme-labeled (β-galactosidase) antibodies (rabbit-mouse) directed against the Fc portion of IgE. The assay is calibrated by the WHO standard for IgE, between 0.35 and 100 kU/l. The cutoff (negative/positive) and the class system remain the same as for the Phadezym RAST.

To express the results, we considered two criteria of positivity: ≥ class 1 (≥0.35 PRU/ml for RAST and ≥0.35 kU/l for CAP), and ≥ class 2 (≥0.71 PRU/ml for RAST and ≥0.71 kU/l for CAP).

CAP inhibition with insect venom

Specific inhibition by homologous venom was applied to 8 of 11 CAP-positive/RAST-negative/ST-negative serum samples, with freeze-dried extracts. Twenty microliters undiluted serum was mixed with 30 µl of various concentrations of venom extracts and incubated for 1.5 h before CAP was run according to the manufacturer's instructions.

Statistical analysis

For the two allergens, we determined the sensitivity, the specificity, and the correlation coefficient of both *in vitro* tests (19).

Results

Among 87 patients with a history of generalized reaction to honeybee or yellow jacket, all but one had

at least one positive test (ST, RAST, or CAP). ST were positive for bee in 63 patients, for yellow jacket in 58, and for both Hymenoptera venoms in 35. Four patients were ST positive only when the concentration of 1 µg/ml of venom was reached: honeybee in two patients and yellow jacket also in two. However, we considered them to be negative, since a large percentage of nonallergic subjects are ST positive with such a venom concentration (1, 8).

The comparison between the results obtained with RAST and those obtained with CAP is shown in Table 1. Concordant results were observed in 90% of the tests for honeybee and 51% for yellow jacket (respectively, 77% and 50%, considering only class 2 or more to be positive).

When we compared the results of both *in vitro* tests obtained in ST-positive subjects, we found that results were more often positive with CAP than with RAST for either of the venoms, indicating a higher sensitivity for CAP (Fig. 1). CAP to honeybee was positive in 98% of the 63 ST-positive subjects and RAST in 95% (95% for CAP and 89% for RAST, considering class 2 or more to be positive). CAP to yellow jacket was positive in 93% of 58 ST-positive subjects and RAST in 40% (84% for CAP and 21% for RAST, considering class 2 or more to be positive).

When both *in vitro* assays were compared in the ST-negative subjects to test the specificity, CAP was more often positive than RAST (Fig. 2). As shown in Table 2, both CAP and RAST to honeybee were positive in five (21%) of the 24 ST-negative subjects (three (12.5%) of the 24 subjects, considering class 2 or more to be positive). For honeybee, the pattern, negative ST/negative RAST/positive CAP was found in five patients (21%). Among these, one had a history of generalized reaction (grade 4) to honeybee (CAP positive in class 1: 0.58 kU/l). CAP and RAST to yellow jacket were both positive in two (7%) of the 29 ST-negative subjects. RAST only was positive in three (10%) and CAP in six (21%). When we considered only class 2 or more to be positive, CAP was the only test to give positive results. It did so in

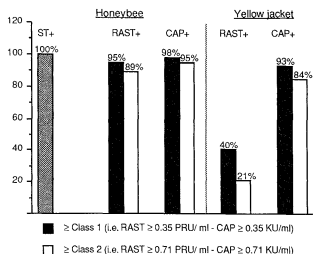


Fig. 1. Sensitivity of RAST and CAP as compared with ST.

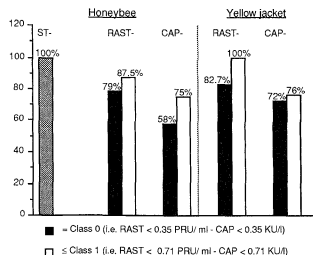


Fig. 2. Specificity of RAST and CAP as compared with ST.

Table 1. Number of concordant and discordant results obtained in comparing the two *in vitro* tests RAST (R) and CAP (C)

Venom	In vitro positivity (≥ class)	No. of cases with					
		Concordant results			Discordant results		
		R-/C-	R-/C+	Total (%)	R+/C-	R-/C+	Total (%)
Honeybee	1	14	64	78 (90%)	1	8	9 (10%)
Yellow jacket	1	20	24	44 (51%)	4	39	43 (49%)
Honeybee	2	21	46	67 (77%)	0	20	20 (23%)
Yellow jacket	2	33	10	43 (50%)	0	44	44 (51%)

seven cases (24%); of these, two patients had a history of generalized reaction (grade 3) to yellow jacket. This hypersensitivity was detected by CAP only (CAP positive in class 2: 1.21 and 1.77 kU/l). Samples with pattern, negative ST/negative RAST/positive CAP were investigated with inhibition assays (Table 3): the response could be inhibited by the homologous allergen in all cases (eight sera including those of the three patients with history of generalized reaction detected by CAP only), in a dose-dependent fashion (honeybee twice and yellow jacket six times).

Among the 87 patients, 53 could identify the offending insect: honeybee in 31 cases and yellow jacket in 22 (Table 4). Among the 31 identifying the honeybee, the history was confirmed by ST and CAP in 30 (97%) and by RAST in 28 (87%). Among the 22 patients identifying the yellow jacket, the history

Table 2. Number of positive RAST (R) or CAP (C) in skin test (ST)-negative subjects

Venom	In vitro pos. (≥ class)	ST-	R+/C+	R+/C-	R-/C+
Honeybee	1	24	5 (21%)	0	5 (21%)*
Yellow jacket	1	29	2 (7%)	3 (10%)	6 (21%)*
Honeybee	2	24	3 (12.5%)	0	3 (12.5%)*
Yellow jacket	2	29	0	0	7 (24%)*

* In one of those, history of generalized reaction to honeybee (CAP 0.58 kU/l=class 1).

** In two of those, history of generalized reaction to yellow jacket (CAP 1.21 and 1.77 kU/l=class 2).

was confirmed by CAP in 19 (86%), by ST in 18 (82%), and by RAST in 9 (41%) only. When we considered only class 2 or more to be positive, the difference between CAP and RAST was more pronounced: for honeybee venom, history was confirmed by CAP in 97% and by RAST in 61% of the cases; for yellow jacket venom, by CAP in 73% and by RAST in 23%.

The coefficient of correlation (*r*) between results obtained with CAP and with RAST was high for both allergens: for honeybee, *r* = 0.925; for yellow jacket, *r* = 0.937.

The interassay reproducibility of CAP was surprisingly high: all positive and negative CAP tests were confirmed except in two cases for yellow jacket. The results of these determinations were, respectively, <0.35 kU/l and 0.38 kU/l for the first serum sample, and <0.35 kU/l and 1.54 kU/l for the second.

Of 40 CAP tests performed with either honeybee or yellow jacket venom in 20 nonallergic subjects, four tests were found to be positive (three subjects, i.e. 15%). One subject had 6.88 kU/l to honeybee and 1.28 kU/l to yellow jacket, the second had 1.05 kU/l to honeybee only, and the third 1.16 kU/l to yellow jacket only.

Table 4. Number of positive skin tests (ST), RAST, or CAP in the 53 patients who identified the offending insect. Number of histories confirmed by three tests

Positive results of	No. of patients with positive results for			No. of histories confirmed by test	
	Honeybee	Honeybee + yellow jacket	Yellow jacket	class 1	class 2
ST ¹	16	14	1	30 (97%)	
RAST ¹	24 (19) ³	4	2 (1) ³	28 (87%)	19 (61%)
CAP ¹	14 (17) ³	16 (13) ³	1 (1) ³	30 (97%)	30 (97%)
ST ²	2	8	10	18 (82%)	
RAST ²	4 (1) ³	5 (3) ³	4 (2) ³	9 (41%)	5 (23%)
CAP ²	2	13 (9) ³	6 (7) ³	19 (86%)	16 (73%)

¹ 31 patients who recognized honeybee as offender.

² 22 patients who recognized yellow jacket as offender.

³ These numbers indicate the results obtained when considering only class 2 or more to be positive.

Discussion

In this paper, we studied the correlation among RAST, CAP, ST, and patient history, with the purpose of evaluating the sensitivity of the new CAP system. Indeed, lack of sensitivity has been recognized as the main drawback of the RAST assay. Because some authors have recommended, when measuring venom-specific IgE, that one consider to be positive only the results of class 2 or more, we also used this criterion of positivity, in addition to that of ≥ class 1, for both RAST and CAP.

On the whole, the CAP assay provided more positive results than did RAST. In subjects with positive ST for honeybee, CAP sensitivity was not significantly higher (98%) than that of RAST (95%) because of the already good sensitivity reached with RAST when performed with honeybee venom. On the contrary, when yellow jacket venom was tested, CAP sensitivity (93%) appeared to be clearly superior to that of RAST (40%). This difference was

Table 3. CAP inhibition assay performed in CAP-positive, RAST-negative, and ST-negative serum samples

Ag μ/ml	CAP (kU/l) to honeybee				CAP (kU/l) to yellow jacket			
100	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	0.8	1.85
50	<0.35	<0.35	<0.35	<0.35	0.44	<0.35	1.01	4.06
25	<0.35	<0.35	<0.35	0.36	0.51	0.37	1.04	3.85
12	<0.35	<0.35	<0.35	0.43	0.54	1.47	1.22	3.57
6	<0.35	0.40	<0.35	0.46	0.59	1.80	1.21	3.99
3	0.35	0.43	0.40	0.48	0.66	2.16	1.27	4.16
1.5	0.37	0.44	0.36	0.48	0.66	1.93	1.18	5.78
0.8	0.39	0.43	0.37	0.46	0.70	2.11	1.39	5.64
0	0.36	0.42	0.47	0.47	0.94	2.01	1.24	6.14
Initial CAP value	0.58	1.1	0.66	0.83	1.21	1.77	2.79	3.32

more pronounced when we considered only results of class 2 or more to be positive. The low sensitivity of RAST for yellow jacket venom found here is in agreement with the results of other studies performed with this method (15, 18, 21). The new CAP system is thus the first assay for the determination of specific IgE capable of reaching a sensitivity clearly superior to that of RAST, and very close to that of the ST, when using yellow jacket venom. The higher sensitivity of CAP, as compared with that of RAST, was recently described in venom-allergic patients (13): 19 of 45 sera with class 1 or 2 in the CAP system were not detected by the RAST system.

In the ST-negative subjects, results were more often positive by CAP than RAST, for both venoms. This might have cast doubt on the specificity of CAP. However, in three of the 53 patients with anaphylaxis when honeybee or yellow jacket was identified as the offending insect, CAP was the only test able to detect the hypersensitivity. Moreover, it is known that, even in a general, unselected population, specific IgE to honeybee or yellow jacket venoms might occasionally be detected: RAST was, for instance, found to be positive – at least for one venom – in 24% and ST in 17% of an unselected population (9). In our control group of nonatopic patients, CAP was found to be positive in 15% of the subjects. Consequently, the finding of specific IgE to venoms in our ST-negative patients presenting with a history of generalized reaction to Hymenoptera venoms is not surprising. The frequent cross-reaction between the allergens of the two venoms (2, 21) could explain the presence of specific IgE for the venoms of both insects, even when ST are positive for one venom only. In addition, in 8 of the 11 sera with the pattern, negative ST/negative RAST/positive CAP, inhibition assays could verify the presence of specific IgE. The response could, in all cases, be inhibited in a dose-dependent fashion by the homologous allergen and was consequently estimated to be immunologically specific. In this regard, the positive CAP results we obtained in ST-negative patients might be considered to be correct and to represent a higher sensitivity of the test rather than a poorer specificity.

In most of the 53 patients who identified the offending insect, the cause of the anaphylactic reaction was confirmed by both ST and CAP: thus, as compared with patient history, the sensitivity of both these tests was shown to be similarly high for both insects. This was not so for RAST, particularly when this test was applied to yellow jacket venom.

In comparison with history and ST results, we found CAP sensitivity to be clearly superior to that of RAST. For both allergens tested, but particularly for yellow jacket venom, CAP represents a

crucial step towards a more accurate diagnostic procedure.

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