

Modified Basophil Degranulation Test in Diagnosis of Bee and Wasp Sting Allergies

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A modified basophil degranulation test proved reliable and easily reproducible in the investigation of 50 patients with allergies to bee and/or wasp stings. Its diagnostic value is comparable to that of the RAST and it is, in addition, simpler, quicker and cheaper. Its disadvantage is that it has to be performed within 24-36 hours after withdrawal of blood.

Key words: basophil degranulation test; bee sting allergy; RAST; wasp sting allergy.

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Ever since Shelley, in 1962, introduced the degranulation of basophils as *in vitro* test for diagnostic purposes, the method has been modified repeatedly (9-13). Yet it has not gained acceptance as a routine procedure because of technical difficulties involved and, as a consequence, the variable reproducibility of the test (2-4, 8).

In 1977 two research teams reported, independently of each other, a further modification of the basophil degranulation test (BDT), using the ability of basophils to stain with toluidine blue only when intact and to lose this property after degranulation. Benveniste et al. (1) employed native blood for their investigation, and the basophils were counted in a Nageotte or Fuchs-Rosenthal chamber. Leynadier et al. (5) used an enriched cell suspension. Following incubation with the specific antigen, they also stained the glutaraldehyde fixed basophils with toluidine blue.

We tried to combine the advantages of the two methods, that is enriching the basophils in the human blood and, after addition of the antigen or the control solution respectively, counting them without previous fixation.

In a first series of investigations we tested patients with house-dust/mite allergies (6). In the present study we employed the BDT in patients with allergies to hymenoptera stings and compared the results with the RAST. Part of these results were published elsewhere (7). Further investigations were to find out whether the BDT is reproducible and how long an interval between withdrawal of blood and examination is permissible to guarantee reliable results.

PATIENTS AND METHODS

Patients

From May 1979 until January 1979, 50 patients with a clinical history of allergic reactions to bee and/or wasp stings and 25 control persons were examined. The controls were 18 patients from the dermatological out-patient department suffering from various skin diseases of non-allergic origin, and seven patients with a house-dust/mite allergy (asthma or rhinitis).

Of the 50 patients with bee and wasp sting allergies, 21 were men, 29 women. The ages ranged between 7 and 75 years (average 39 years). In five patients, the history was not very reliable. Ten patients had been allergic for less than 6 months, five for 6 months to 5 years and the remaining 35 for 3–26 years (average 7 years). Most of the patients showed generalized symptoms such as asthma and/or urticaria and angioneurotic oedema, collapse and other signs of anaphylactic shock. At the time of the BDT test, 38 of the 50 patients were on a course of desensitization with bee and/or wasp whole body extract, five patients for less than 2 years, 33 for more than 2 years (average 9 years).

RAST

The RAST test were performed in the laboratory of the Paediatric University Clinic (Prof. Girard) according to the Pharmacia standard method. Up to the completion of this investigation, no wasp venom extract was available, therefore the RAST was only carried out with the Pharmacia bee venom extract.

Basophil degranulation test

Twice 10 ml blood are drawn into a heparinized vacutainer (Becton-Dickinson 4512 Lithium heparin 143 units USP) and centrifuged at 500 *g*. The supernatant plasma and 0.2 ml of the adjacent red cell layer are collected into small plastic tubes (Falcon tissue culture tube 2085, 12 × 75 mm), thoroughly mixed and centrifuged again for 5 min at 650 *g*. The supernatant serum is then removed to about 1 cm above the cell layer and the remainder mixed on the rocking mixer. 100 μ l each of this suspension are transferred with a micropipette into six plastic tubes (Falcon 2085). 5 μ l each of NaCl and five antigen solutions (bee: scratch test solutions Allpyral and Purex (whole insect body extracts), Forapin Mack (bee poison preparation) concentration D; wasp: scratch test solutions Allpyral and Purex) are added.

The test tubes are incubated at 37°C for 20 min and placed on the rocking mixer for 5–10 s. 20 μ l of each solution are withdrawn into fresh tubes, mixed with 180 μ l toluidine blue (0.025% toluidine blue in 30% ethanol, brought to pH 3.5 with 4 μ l/ml glacial acetic acid) and placed on the rocking mixer for 2–3 s. With a Pasteur pipette, both sides of a Neubauer haematocytometer chamber are then filled. The intact basophils are counted, their plasma appears distinctly reddish compared with the other leukocytes. In the other leukocytes, only the nucleus stains faintly blue. If not enough basophils are present, the test tube with the buffy coat and the antigen is centrifuged once more at 650 *g* for 5 min, 200 μ l of the supernatant plasma are removed, and the remainder is examined.

For counting under the microscope, a magnification of 10 × 10 is used. The reduction in the number of stainable, therefore still intact, basophils after addition of the antigen is expressed as percent of the values in the control tubes:

$$\frac{n \text{ basophils in cont. sample} - n \text{ basophils in test sample}}{n \text{ basophils in control sample}} \times 100$$

Results were only evaluated when at least 25 basophils could be counted in the control samples incubated with NaCl.

The test results are marked as follows:

degranulation of 0–20% = negative –
degranulation of 21–25% = doubtful ±
degranulation of 26–50% = positive 1 +
degranulation of 51–75% = positive 2 +
degranulation of over 75% = positive 3 +

The expenditure of time for the complete test with five antigens was 1 hour.

The reproducibility, i.e. the standard deviation of the method, was investigated in three patients and three control persons. From each incubated plasma, 3 times 20 μ l were removed, stained and counted separately.

To assess how long an interval between withdrawal of the blood and performance of

Table 1

Results of RAST and BDT with bee and wasp extracts in 50 patients and 25 control subjects

Test	RAST bee	BDT bee	BDT wasp	BDT bee	BDT wasp
No. of subjects tested*	45** (P)	50 (P)	50 (P)	25 (C)	25 (C)
<i>Results</i>					
Negative	22%	34%	48%	88%	88%
Doubtful	13%	6%	6%	4%	4%
Positive	65%	60%	46%	8%	8%

* (P), patients. (C), controls.

** In 5 patients, RAST could not be performed for various external reasons.

Table 2

Comparison of the results of BDT and RAST with bee extracts in 45 patients allergic to hymenoptera stings

	RAST positive**	RAST negative
BDT positive*	45%	20%
BDT negative	20%	15%

* More than 25% degranulation

** Class no. 2-4.

the test is permissible, parts of 10 different blood samples were examined after 1 hour and after 1, 2 and 3 days. The samples were kept at room temperature.

RESULTS

Table 1 lists the overall test results in 50 patients with a probable clinical history of allergy to hymenoptera stings and in 25 control subjects.

Table 2 gives the correlation between RAST and BDT in the 50 patients with a history of allergic reaction to hymenoptera stings. There is a correlation of 60% between the two methods. Twenty per cent show a positive reaction to the RAST only, another 20% to the BDT only.

Table 3 illustrates the reproducibility of the BDT. There are no serious differences in the

six individual tests performed with the same blood sample. The standard deviation for the total of the tests is 1.74.*

Table 4 shows that the BDT may still be performed on the day after withdrawal of the blood. The number of basophils decreased less than 20% after 1 day in eight out of the 10 samples.

DISCUSSION

Our investigations in hymenoptera sting allergies demonstrate that the modified BDT yields results which are comparable with the RAST. It must be stressed, however, that with the RAST the "surplus" of non-cell-fixed IgE

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Table 3
Number of basophils in three patients allergic to hymenoptera stings and three control subjects after incubation of the blood with NaCl, three bee extracts and two wasp extracts. Results of three independent evaluations

	n	NaCl	Bee			Wasp	
			Allpyral	Purex	Forapin	Allpyral	Purex
Allergic patients	45	22, 23, 21	15, 16, 17	7, 10, 8	23, 22, 20	22, 24, 22	24, 22, 24
	47	21, 20, 24	5, 6, 4	25, 22, 23	21, 18, 19	25, 24, 20	22, 20, 22
	48	35, 36, 33	23, 24, 22	35, 38, 32	36, 37, 35	22, 25, 23	23, 22, 24
Controls	11	18, 20, 22	19, 22, 20	21, 24, 18	15, 16, 18	16, 18, 20	16, 17, 19
	16	36, 33, 34	31, 35, 30	40, 38, 33	32, 37, 33	34, 36, 34	32, 32, 35
	20	19, 18, 20	17, 19, 19	18, 20, 20	19, 19, 22	17, 18, 19	20, 22, 23

Table 4
Number of basophils 1 hour, and 1, 2 and 3 days after withdrawal of blood

Patients No.	1 hour	1 day	2 days	3 days
1	19	18	17	7
2	51	56	34	8
3	30	19	14	—
4	51	30	33	23
5	14	14	12	—
6	30	25	13	—
7	40	40	34	—
8	35	30	22	—
9	68	65	38	7
10	25	20	13	—

in the sera is measured, whereas with the BDT the specific IgE, which are already fixed on cells, are assessed. IgE have a great avidity for the receptors of basophils and mast cells, and their half-life as tissue-fixed IgE is weeks and months, whereas in the serum it is only about 2½ days. Because of this great avidity and long half-life, basophils may still be densely covered with specific IgE and therefore give a positive BDT, even when the quantity of "surplus" IgE in the serum has sunk below the level detectable with the RAST.

The fact that in most patients with positive BDT and negative RAST the allergic reaction had taken place more than 2 years previous to the test, would be in line with such a hypothesis. But one has also to keep in mind that the extracts used for the RAST were not identical with those used in the BDT and therefore some divergent results had to be expected.

The question remains open whether part of the negative results (BDT and RAST) in patients with a sure history of an allergic reaction to hymenoptera stings are due to the desensitization, which has been carried out sometimes for years, and that IgE have therefore in the course of time sunk below measurable values.

Basophils are very unstable cells, and many unspecific factors may induce degranulation. The results in Table 3 demonstrate, however,

that the test is easily reproducible and that the basophils only rarely show an unspecific degranulation (see Table 1, last column, i.e. maximum 12%). Furthermore it is shown in Table 4 that the stability of the basophils under the described test conditions is relatively good and that spontaneous degranulation renders the test impossible only about 36 hours after the withdrawal of blood.

The reliability of the test as demonstrated in Tables 3 and 4 make it suitable for routine examination, particularly as an alternative to the RAST in smaller laboratories, which have no access to radioactive test equipment and the corresponding counters.

Advantages and disadvantages of the BDT may be summed up as follows:

Disadvantages:

- the test must be performed within a certain time after withdrawal of blood, i.e. within 24–36 hours.

Advantages:

- simple technique, it can be carried out in every laboratory;
- the result is available within 1 hour;
- specific, sensitive, reproducible, yet no exposure of personnel to X-rays, no contamination of environment; no expensive equipment, therefore:
- cheap (one test with five different antigens costs about 15 Sf., the RAST with one single antigen costs 20–25 Sf.).

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