

Original article

Comparison of basophil activation tests using CD63 or CD203c expression in patients with insect venom allergy

Background: Flow cytometric basophil activation tests have been developed as cellular tests for *in vitro* diagnosis of IgE-mediated reactions. Different activation markers (CD63 or CD203c) with distinct ways of regulation have been used after stimulation with various allergens.

Objective: It was the aim of the present study to compare basophil activation tests by measuring both CD63 and CD203c upregulation in patients with insect venom allergy.

Materials and methods: 43 patients with a history of insect venom anaphylaxis were examined. A careful allergy history was taken, and skin tests and determination of specific IgE-antibodies were performed. Basophil activation tests (BAT) using CD63 or CD203c expression were done after stimulation with different concentrations of bee and wasp venom extracts. 25 healthy subjects with negative history of insect venom allergy were studied as controls.

Results: The CD203c protocol showed a slightly higher sensitivity than the CD63 protocol (97% vs. 89%) with regard to patients' history. The magnitude of basophil response was higher with CD203c in comparison to CD63 for both insect venoms. Specificity was 100% for the CD63 protocol and 89% for the CD203c protocol with regard to controls with negative history and negative RAST.

Conclusion: These results support the reliability of basophil activation tests using either CD63 or CD203c as cellular tests in the *in vitro* diagnosis of patients with bee or wasp venom allergy with a slightly higher sensitivity for the CD203c protocol.

**B. Eberlein-König^{1,2}, R. Varga^{1,2},
M. Mempel¹, U. Darsow¹,
H. Behrendt², J. Ring¹**

¹Department of Dermatology and Allergy
Biederstein, Technical University Munich;

²Division 'Environmental Dermatology and
Allergology' GSF/TUM, Neuherberg-Munich,
Germany

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Priv.-Doz. Dr Bernadette Eberlein-König
Klinik und Poliklinik für Dermatologie und
Allergologie am Biederstein
Technische Universität München
Biedersteiner Str. 29
D-80802 München
Germany

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Flow cytometric basophil activation tests (BAT) have been developed as cellular tests for *in vitro* diagnosis of immunoglobulin (Ig)E-mediated reactions. The aim of the present study was to compare BAT by measuring both CD63 and CD203c upregulation in patients with hymenoptera allergy.

Materials and Methods

Skin tests and determination of specific IgE antibodies were performed in 43 patients. BAT using CD63 (BASOTEST®, Orpegen Pharma, Heidelberg, Germany) or CD203c expression (mAb97A6, Beckman Coulter, Krefeld, Germany) were performed after stimulation with different concentrations of bee and wasp venom extracts (Reless®, ALK SCHERAX, Hamburg, Germany). For the CD63 protocol a stimulation buffer containing interleukin (IL)-3 was used, in the CD203c protocol washing solution instead. Twenty-five healthy subjects with negative history of insect venom allergy served as controls.

Results

In patients mean percentage of activated basophils using CD203c was slightly higher (not significant) than using CD63 in the negative (phosphate-buffered saline, PBS) and positive controls (*N*-formyl-methionyl-leucyl-phenylalanine) as well as at all insect venom concentrations (Fig. 1). In the control group without specific IgE antibodies (*n* = 18), CD63 expression was below the cut-off (15% after receiver–operator characteristic analysis) after incubation with bee or wasp venom in all cases, CD203c expression in 16 of 18 cases (wasp venom at 1 µg/ml; cut-off: 14% after receiver–operator characteristic analysis). In the control group with specific IgE antibodies (*n* = 7), mean percentage of CD63 or CD203c expression was not above the cut-off after incubation with bee venom. After incubation with 0.1 µg/ml or 1 µg/ml wasp venom mean expression of basophil activation markers were above the cut-offs (CD63: 18.2%, 19.7%; CD203c: 18.6%, 21.5%).

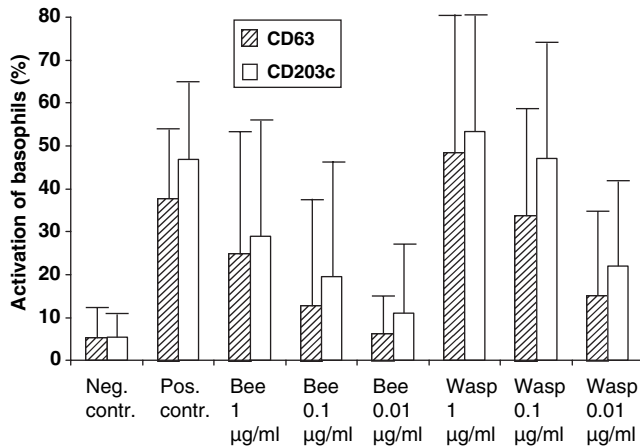


Figure 1. Percentage of CD203c and CD63 expression of basophils after incubation with buffer (negative control), N-formyl-methionyl-leucyl-phenylalanine (FMLP) (positive control) and three bee or wasp venom concentrations in patients with a history of anaphylactic reactions to insect stings (mean $\pm$ SD; $n = 43$).

Of the 43 patients, 31 (72%) had a congruent result in both BATs irrespectively of history, skin test and determination of specific IgE antibodies. Sensitivity for BAT using CD63 expression was 89%/88%/83%, for the BAT using CD203c expression 97%/73%/66% calculated with regard to clinical history/skin test/radioallergosorbent test (RAST). Specificity for BAT using CD63 expression 100%, for BAT using CD203c expression 89% with regard to controls with negative history and negative RAST. Correlation coefficient was 0.85 for the highest bee venom concentration and 0.69 for the highest wasp venom concentration.

Discussion

This study shows that BAT with CD63 or CD203c are sensitive and reliable cellular *in vitro* tests in the diagnosis of insect venom allergy. Sensitivity of BAT using CD63 has been reported between 92% and 100% respectively (1–3). Using a CD63 BAT without preincubation with IL-3, 91.3% tested positive in the BAT for bee venom and 85.3% for wasp venom (4) suggesting that preincubation with IL-3 increases the sensitivity in this test. Using CD203c as an activation marker one study showed a sensitivity of 91%, another of 92% (5, 6).

Data in patients with latex allergy showed better results with the BAT using CD203c than using CD63 (sensitivity 75% vs 50%), but these results were controversially discussed by other authors. In patients with anaphylactic reactions to muscle relaxants BAT with CD63 was favourable to CD203c (sensitivity 79% vs 36%).

In previous studies specificity varied between 80% and 100% (1, 2, 4–6), but part of the controls also had elevated specific IgE values. In the present study, specificity for controls with negative history and detection of specific IgE antibodies was 86% for the CD63 protocol and 71% for the CD203c protocol.

Though in our hands both tests are useful cellular tests for patients with insect venom allergy general recommendations regarding preference of one test cannot be given at the moment because of the contradictory results of other studies with different allergens. This might be due to different protocols, e.g. for the CD203c protocol a triple-staining flow cytometric protocol was used. Also these investigators did not perform a preincubation with IL-3 in the CD63 protocol.

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