

Basophil activation test displays a higher CDsens compared to histamine release assay and is capable of diagnosing patients with a low basophil number

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Aim:

Basophils activation in response to allergen stimulation can be used as a surrogate marker of mast cell activation and thus utilized for allergy diagnosis. Basophil activation test (BAT), histamine release assay (HR) and passively sensitized basophil histamine release assay (passive HR) are all methods relying on this principle. In this study we compared the performance of BAT, HR and passive HR.

Method:

Blood samples were drawn from non-allergic (n=14) and peanut allergic (n=11) subjects following informed consent. Blood samples were subjected to BAT, HR and passive HR. **BAT:** blood was incubated with anti-IgE or peanut extract for 30 min at 37 °C and concurrently stained with anti-CD63, anti-CD123 and anti-CD193. Erythrocytes were lysed and cells were washed, fixed and acquired on the flow cytometer. The blood was processed within 4 hours of collection and basophils were gated as CD123⁺CD193⁺ cells of single cells. **HR:** blood and stimulant (anti-IgE, peanut extract or phorbol 12-myristate 13-acetate + ionomycin (PMA/I)) were added to glass fiber-coated microtiter plates (HR-plates) and incubated at 37 °C for 60 min. Released histamine was detected using a fluorescence method, according to the manufactures' procedure (RefLab). **Passive HR:** Buffy coats were screened for reactivity to anti-IgE, PMA/I, inhalation and food allergens. A buffy coat with high histamine release to anti-IgE, PMA/I and no histamine release to inhalation or food allergens were selected. The buffy coat was incubated with 10 pg/ml of IL-3 overnight at 5 °C. Bound autologous IgE were removed from the surface of basophils by acid treatment. Cells were washed and passively sensitized by adding serum from the study participants and incubated at 37°C for 1h. Subsequently, cells were added medium, anti-IgE, or peanut extract in a HR-plate and incubated at 37°C for 1h. HR- Plates were developed and the released histamine was quantified as mentioned above.

Results:

BAT, HR and passive HR was performed on peanut allergic subjects (peanut sIgE >3.5 kU/l, ImmunoCAP) and age + gender matched non-allergic controls. CDsens was calculated from each dose-response curve as the inverted peanut extract concentration eliciting 50 % of maximum response times 100. CDsens was significantly higher in BAT (103 ± 16.9) compared to HR (25.7 ± 11.1) and passive HR (12.2 ± 1.8) demonstrating that the peanut extract concentration needed to reveal a reaction is lower in the BAT. Comparing the diagnostic performance of the test showed that BAT was able to identify a positive reaction to peanut in 11 out of 11 peanut allergic individuals, whereas HR identified 10 and passive HR 9. However when combining HR and passive HR all 11 peanut allergic patients could be shown to react. The one peanut allergic subject who did not react in HR was due to a very low number of basophils and hence very low or no detection of histamine release. One peanut allergic subject who did not react in passive HR also needed a very high concentration of peanut extract to appear positive in BAT and HR. The number of inconclusive tests due to no reaction to anti-IgE or peanut extract stimulation was 2 for BAT, 5 for HR and 1 for passive HR. None of the non-allergic participants consistently reacted to peanut extract stimulation in the three cellular tests.

Conclusion

Basophil activation test display a higher CDsens and is capable of diagnosing patients with low basophil number and has less inconclusive test results compared to the histamine release assay.