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Basophil activation test: do not lose control

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Basophils, as mastcells, represent the military arm of IgE-mediated immune response. Plasma cells-secreted IgE sensitize mast cells and basophils by binding to FcεRI. Subsequent exposure to the allergen leads to the activation of these cells by bridging/cross-linking of FcεRI receptors. The release of various mediators such as histamine, leukotrienes, prostaglandins and citokines is responsible of cutaneous symptoms (e.g., urticaria or angioedema), respiratory symptoms (e.g., asthma), and in some cases anaphylaxis.

Basophil Activation Test (BAT) is an amazing "*in vitro*" method, able to simulate the encounter between basophils and the allergen and to assess the subsequent cellular activation by analising the expression of activation markers on cell surface by flow cytometry. CD203c (a member of ectonucleotide pyrophosphatase/phosphodiesterase family) and CD63 (a protein associated with intracellular vesicles membranes) are the most reliable basophils activation markers presently available (1-3).

The test is performed using whole blood rather than isolated leukocytes, due both to the simpler and faster manipulation of the method, but also for the belief that leaving basophils in their natural environment ensures a better functionality (4).

Until ten years ago, BAT was used as a diagnostic method in drug allergy, with controversial results in terms of sensibility and specificity of different drugs evaluated.

During the last years, several scientists have shown the usefulnes of BAT as a functional assay, able to analyse the cellular activation threshold toward an allergen. In this way, BAT has been used to monitor the development of tolerance in children with food allergy before oral challenges (5,6). Other data showed the usefulness of BAT in the evaluation of tolerance induction in venom-allergic patients treated with specific immunotherapy (SIT), in order to predict the outcome of SIT and clinical sensitivity of the patient (7).

In the light of this novel use of BAT in allergy diagnosis and monitoring, the paper by Pereira Santos *et al.* about "the expres-

sion of FcεRI, IgE on basophils and dendritic cells in association with basophil function in two patients with severe allergic asthma treated with Omalizumab" appearing in this issue of European Annals of Allergy and Clinical Immunology, is very current and interesting. In this paper, the authors describe the evolution of IgE and FcεRI expression on different cell types, and changes in basophil activation following allergen stimulation before and during successful omalizumab treatment in two severe mite-allergic asthmatic patients.

After omalizumab treatment, the authors observed significant reductions of surface IgE and FcεRI expression on basophils, myeloid dendritic cells and plasmacytoid dentritic cells. By performing BAT, following mite stimulation, they observed a parallel trend with reduction in basophil reactivity in both patients during the first month, with additional reductions between months 1 and 12 of omalizumab treatment.

These data raise the possibility that BAT could be indicative of a complete, incomplete or non-response to omalizumab.

Whether BAT might also predict a possible relapse occurring after omalizumab discontinuation, represents a fascinating question.

In the present issue of European Annals of Allergy and Clinical Immunology, the paper by Pereira Santos *et al.* leads us to some technical considerations, particularly concerning the evaluation of basophil reactivity after allergen stimulation.

One of the crucial points in the sequential analyses performed to evaluate changes in basophil reactivity at different time steps (days or months) during a drug or SIT treatment, is a correct evaluation of the intrinsic cellular reactivity, which can vary over time. Basophil intrinsic reactivity may change from day to day and month to month. For this reason it is extremely important that a positive control able to check basophil specific immunologic intrinsic *IgE mediated* response is used, along with a negative control when BAT is performed. Monoclonal antibody anti FcεRI represents the best one, because it is able to induce the maximum FcεRI-mediated cellular activation (4).

Another crucial point is represented by the observation that basophils change their intrinsic reactivity over time. One can observe different values of anti-FcεRI-induced basophil activation if BAT is carried out in different times. For this reason, it is crucial to evaluate basophil activation after allergen stimulus by taking basophil intrinsic reactivity into account. The best evaluation of specific allergen basophil activation is performed by applying the following formula: [allergen basophil activation (%) / anti-FcεRI (%)] x100. This formula allows to relate BAT result after allergen stimulus with intrinsic basophil reactivity at the time when the test was performed, and to standardize the data. Clearly, a basophil activation of 45% after allergen stimulation in a patient showing a positive control of 50% has to be evaluated in a different way from the same percentage of activation if the same patient shows a positive control of 80% in another moment of his life.

In conclusion, BAT is a useful method to evaluate basophil reactivity and sensitivity to an allergen, and could be probably used as a biomarker in monitoring drug and/or SIT treatment in IgE-mediated diseases. However, even if you are struck by the charm of this test, remember… NOT TO LOSE CONTROL.

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