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Basophil activation test: handle with care

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The letter to the Editor by Dr Chirumbolo highlights the major pitfalls in basophil activation test (BAT) performance.

In my view, basophil activation test should be considered as an additional clinical tool in the diagnosis of allergy and not the "absolute truth".

Basophils play a pivotal role as effector cells in allergic disease, despite representing a small population of peripheral leucocytes. The earliest assessment of human basophil activation was based on the measurement of *ex vivo* histamine and leukotriene release, but the poor handling of laboratory methods and the discovery of basophil surface activation markers induced most scientists to use flow cytometry. Following this experience, different authors were able to demonstrate a good correlation between BAT and histamine releasing test in analyzing basophil activation after allergen stimulation (1,2).

However, the use of flow cytometry implies that basophil identification markers represent one of the major issues in BAT performance, but, as Chirumbolo published in his commentary on Cytometry in 2014 (3), I believe that the contemporary targeting of CCR3 (CD193) and CD3 in a flow cytometry system is one of the methods to well discriminate basophils from leucocyte pool, considering that CCR3 is expressed on basophils and eosinophils (easily discriminable for different SSC) and on lymphocytes which expressed CD3 molecule, which is absent on basophils.

CD63 still remain the most important marker of basophil activation (4,5). It was discovered in 1990 by Edward Knol and coworkers, and its expression is closely related to the phenomenon of basophil degranulation (1), even if in a number of cases the expression of CD63, the production of LTC4 and histamine release may be entirely dissociated, suggesting that although they are often correlated in clinical practice, the various outcomes may occur independently (6,7).

CD203c is another accredited marker of basophil identification and activation, as it is constitutively expressed on cell surface. Basophil activation results in an upregulation of the molecule with continuous increase in fluorescent CD203c intensity and this phenomenon limits the gating between non activated (low CD203c positive) and activated basophil.

Conversely, the CD63 expression on basophil surface is an all-or-nothing phenomenon, allowing to better discriminate activated from resting basophils. Nevertheless, in some cases it could be difficult to identify a threshold between resting and activated basophils, because of a number of variables that will determine individual basophil outcomes (i.e. total IgE receptor cell surface density; ratio between membrane-bound allergen-specific IgE versus total IgE; intrinsic cellular sensitivity of basophils, evaluated as number of IgE molecules required for 50% of maximal cellular responses; presence of specific IgG competing with IgE; etc.).

For these reasons, we can establish a presumable cutoff point for each allergen, by setting up a receiver operating characteristic (ROC) curve to obtain optimal sensitivity and specificity, as we usually do with other cellular assays.

The possibility of non-responder individuals should be taken into account. The percentage of non-responders is usually near or below 10%, and in these cases the BAT is not available as diagnostic tool and data will not be included in the ROC curve.

The use of an anti-FceRI, as positive stimulation control, is an easy tool to assess basophil reactivity and to establish non-responders individuals. f-Met-Leu-Phe (fMLP) molecule is an another useful stimulation control in BAT because it acts via G-protein-coupled receptor that activates MAPK pathways and phospholipase C without following an IgE mediated activation, and it can be applied as a tool to assess viability of the cells.

In summary, I share the doubts expressed by Dr Chirumbolo in his letter, and I also hope that the use of new basophil identification and activation markers will improve the method sensitivity. At the moment, I think that by using current methodologies in basophil activation analysis associated with strict criteria of data evaluation we can get useful information that, added to other diagnostic tests and compared with clinical observations, will allow a better understanding of patient's disease.

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