

S. CHIRUMBOLO

Is basophil activation test (BAT) really useful for allergy diagnosis?

Department of Medicine, Unit of Geriatrics, University of Verona, Italy

Corresponding author

Salvatore Chirumbolo
University Laboratory of Medical
Research (LURM)
Unit of Geriatric Medicine, Room 41
Department of Medicine University of
Verona, Italy
Policlinico GB Rossi
Piazzale L.A. Scuro 10, 37134 Verona, Italy
Phone: +39 045 8128 456
E-mail: salvatore.chirumbolo@univr.it

Dear Editor,

both the paper by Pereira Santos et al and the Editorial on the latest issue of this Journal, stressed on the essential, fairly irreplaceable role of basophil activation test (BAT) in allergy diagnosis (1,2). Certainly, BAT is a cell-based test and in this sense it should give fundamental insights about the role of these circulating leukocytes in hypersensitivity reactions, chronic allergy and anaphylaxis (3). However, as many other analytical assays, even BAT has its Achilles's heel.

Although a great deal of efforts have been attempted in order to improve the diagnostic potential of BAT, principally regarding its flow cytometry (FC) approach (4,5), operators are still using CD63% as the main tuner of the analytical decision if the activated basophils in BAT are signalling an allergic response. CD63% is an analytical algorithm that raised some criticism and concern principally because of its relationship with an arbitrary threshold, closely dependent on the resting, non activated state of basophils, used as a negative reference control (6). Tracing a landmark of the resting, full activable basophil in a BAT is particularly difficult and attempts were suggested to address this cumbersome issue in BAT interpretation (7,8). In

few words, it is practically impossible to set basophil activation on a standard resting population, because of the many reasons related to pre-analytical handling, frequency of releaser or non releaser subjects within the population, intrinsic variability due to CD63 vesicle endowment.

The paper of Pereira Santos evaluated single asthmatic patients treated with omalizumab, and raised another fundamental issue in the correct use of BAT in allergy. Xolair® Omalizumab is able to reduce both serum IgEs and FcεRs on basophils and dendritic cells (DCs), therefore, according to the Authors, a CD63-based BAT should probe the effect of omalizumab on basophil reactivity, following the anti-Cε3-IgE MoAbs treatment (1). However, the same Authors addressed the critical issue that BAT does not match completely the effect of omalizumab on a IgE-mediated response. The intrinsic variability in the allergic response performed by basophils, particularly addressed in the Editorial (2), might hinder the presumptive IgE/CD63 linear relationship, as expected if considering the close relationship between allergic response and CD63 membrane up-regulation. Yet, BAT is evaluated through the behaviour of a marker that does not depend exclusively on the membrane FcεRI/IgE complex expression

and receptor recycling mechanism (9,10). Pereira Santos and colleagues did not deepen how a marked reduction in FcεR1 on DCs may affect BAT performance in patients treated with omalizumab, furthermore they showed both myeloid DCs and plasmacytoid DCs with only using CD123 and HLA-DR markers, without employing CD11c (11,12), and probably they should explain better DCs capture in FC, if a CD123/HLA-DR gate on the CD45^{dim} area allows to capture basophils, as previously reported (13). A decrease in serum IgE does not appear to be linearly related to IgE-mediated response in basophils. The use of an anti-FcεRI in an FC approach seems intriguing, in order to set basophils about their membrane receptor binding ability, but this attempt may result in cumbersome and time consuming procedures. An interesting suggestion might come apparently from using an anti-IgE monoclonal antibody able to react with circulating IgEs and membrane IgE/CD23 complexes, such as the mAb 8D6 (14). In this circumstance, it is possible to deepen the role of FcεRII (CD23), which is not expressed by basophils, on the allergy mechanism and BAT when a monoclonal humanized anti-IgE antibody is used. The mAb 8D6 can compete with omalizumab in binding the conformational epitope of IgE-CH3 domain, and as omalizumab it does not interact with membrane FcεRI-bound IgEs but can bind FcεRII-IgEs (14). Although 8D6 does not activate basophils *in vitro* (14), its use may be fundamental in assessing the role of circulating IgEs in omalizumab-treated patients and in their related BAT. Activated B cells express CD23, the isoform CD23a, the cell recycling of which is strictly related with HLA-DR and antigen presentation and moreover CD23 is fundamental to enhance the IgE-antigen presentation to T-cells (15). IgEs protect CD23 from cleavage and stabilize its intracellular recycling, then its evaluation might give insights into the involvement of B/T cells relationship in allergy and shed a light on the puzzling issue if circulating IgEs, escaped to omalizumab, may be able to elicit an immune response, as recent reports have shown that omalizumab is able to target B-cells membrane IgEs (15-17). Yet, the role of CD23 should be fundamental in ensuring a residual IgE-mediated response in the absence of circulating IgEs and their interactions with high affinity FcεRs. However, we cannot be sure, in this regard, if MAb 8D6 may give a sound, further contribution in the comprehension of the immune response of omalizumab-treated patients, aside from the direct responsiveness of FcεRI-bearing cells, such as basophils or mast-cells, due to the complexity of the immune response involving B and T cells.

Notwithstanding, when CD63 is used as the main marker to evaluate basophil response to omalizumab treatment, BAT does not appear to well correlate with FcεRI (PT2, **figure 1**), despite the rapid decrease of IgE high affinity receptor following one month treatment (1). This observation may raise some concern about the diagnostic capability of BAT in omalizumab follow up

by the simple detection of CD63%. Other tetraspanins, such as CD81/TAPA-1, might be considered or added as possible molecules more closely related to the FcεRI-IgE mediated response in basophils (18,19). Yet, the role of other well known activation markers such as CD203c, CD164, CD193 (CCR3), should be better evaluated in BAT used to investigate omalizumab therapy (20), probably this issue should add more interesting insights to the evidence recently reported (1).

The quality of basophil response in a BAT depends on a huge amount of unpredictable factors, including the complexity of the acquired immune response involving B cells. A recent paper suggested the role of CD63-containing granules in assessing the baseline level of “responsiveness” (7) and in this sense even the interesting observation of Maietta meets this issue (2), though mainly related to IgE receptors. Then, the major concern may be considered the search for a zero point, which any activation event should be referred to.

In my opinion, this zero point is restricted to the particular circumstance and individual’s ability to respond and cannot be completely standardized. Apparently, this circumstance appears to prevent the setting of an optimal cut off for CD63 up-regulation. And actually this seems to be the major criticism to address, in order to optimize BAT in allergy (6). Most probably, the researcher should look for further activation markers, which exhibit a closer relationship with FcεRI activity and turn over, by evaluating MFIs and optimal FC algorithms on fluorescence (without naïve percentage assumptions) or ultimately by improving the predictive and diagnostic value of CD63 marker. BAT might yet allow researchers in the achievement of important data about basophil involvement in allergy, immunotherapy and anaphylaxis, although investigation needs more insights.

References

1. Pereira Santos MC, Campos Melo A, Caetano A, Caiado J, Mendes A, Pereira Barbosa M, Branco Ferreira M. Longitudinal study of the expression of FcεRI and IgE on basophils and dendritic cells in association with basophil function in two patients with severe allergic asthma treated with Omalizumab. *Eur Ann Allergy Clin Immunol.* 2015;47(2):38-40.
2. Maietta G. Basophil activation test: do not lose control. *Eur Ann Allergy Clin Immunol.* 2015;47(2):36-7.
3. Siracusa MC, Kim BS, Spergel JM, Artis D. Basophils and allergic inflammation. *J Allergy Clin Immunol.* 2013;132(4):789-801.
4. Eberlein B, Hann R, Eyerich S, Pennino D, Ring J, Schmidt-Weber CB, Buters J. Optimizing of the basophil activation test: Comparison of different basophil identification markers. *Cytometry B Clin Cytom.* 2014;17. doi: 10.1002/cyto.b.21203.
5. Chirumbolo S, Ortolani R, Vella A. CCR3 as a single selection marker compared to CD123/HLADR to isolate basophils in flow cytometry: some comments. *Cytometry A.* 2011;79(2):102-6.
6. Chirumbolo S. Major pitfalls in BAT performance may be caused by gating protocols and CD63% cut off evaluation. *Cytometry A.* 2014;85(5):382-5.

7. Chirumbolo S. Monitoring of CD63% in basophil activation test and suggested new parameters for allergy diagnosis. *Inflamm Res*. 2012;61(2):171-6.
8. Chirumbolo S. Basophil activation test in allergy: time for an update? *Int Arch Allergy Immunol*. 2012;158(2):99-114.
9. Chirumbolo S, Olivieri M. Increase in human basophils IgE-mediated stimulation by omalizumab: a role for membrane FcγRs? *J Allergy Clin Immunol*. 2014;133(5):1493-4.
10. MacGlashan D Jr. Expression of CD203c and CD63 in human basophils: relationship to differential regulation of piecemeal and anaphylactic degranulation processes. *Clin Exp Allergy*. 2010;40(9):1365-77.
11. Rovati B, Mariucci S, Manzoni M, Bencardino K, Danova M. Flow cytometric detection of circulating dendritic cells in healthy subjects. *Eur J Histochem*. 2008;52(1):45-52.
12. Abeles RD, McPhail MJ, Sowter D, Antoniadis CG, Vergis N, Vijay GK, Xystrakis E, Khamri W, Shawcross DL, Ma Y, Wendon JA, Vergani D. CD14, CD16 and HLA-DR reliably identifies human monocytes and their subsets in the context of pathologically reduced HLA-DR expression by CD14(hi) / CD16(neg) monocytes: Expansion of CD14(hi) / CD16(pos) and contraction of CD14(-lo) / CD16(pos) monocytes in acute liver failure. *Cytometry A*. 2012;81(10):823-34.
13. Chirumbolo S, Vella A, Ortolani R, De Gironcoli M, Solero P, Tridente G, Bellavite P. Differential response of human basophil activation markers: a multi-parameter flow cytometry approach. *Clin Mol Allergy*. 2008;16:6:12.
14. Shiung YY, Chiang CY, Chen JB, Wu PC, Hung AF, Lu DC, Pan RL, Chang TW. An anti-IgE monoclonal antibody that binds to IgE on CD23 but not on high-affinity IgE.Fc receptors. *Immunobiology*. 2012;217(7):676-83.
15. Karagiannis SN, Warrack JK, Jennings KH, Murdock PR, Christie G, Moulder K, Sutton BJ, Gould HJ. Endocytosis and recycling of the complex between CD23 and HLA-DR in human B cells. *Immunology*. 2001;103(3):319-31.
16. Lee WT, Rao M, Conrad DH. The murine lymphocyte receptor for IgE. IV. The mechanism of ligand-specific receptor upregulation on B cells. *J Immunol*. 1987;139:1191-8.
17. Chan MA, Gigliotti NM, Dotson AL, Rosenwasser LJ. Omalizumab may decrease IgE synthesis by targeting membrane IgE+ human B cells. *Clin Transl Allergy*. 2013;2;3(1):29.
18. Fleming TJ, Donnadieu E, Song CH, Laethem FV, Galli SJ, Kinet JP. Negative regulation of Fc epsilon RI-mediated degranulation by CD81. *J Exp Med*. 1997;20;186(8):1307-14.
19. Agis H, Füreder W, Bankl HC, Kundi M, Sperr WR, Willheim M, Boltz-Nitulescu G, Butterfield JH, Kishi K, Lechner K, Valent P. Comparative immunophenotypic analysis of human mast cells, blood basophils and monocytes. *Immunology*. 1996;87(4):535-43.
20. Gernez Y, Tirouvanziam R, Yu G, Ghosn EE, Reshamwala N, Nguyen T, Tsai M, Galli SJ, Herzenberg LA, Herzenberg LA, Nadeau KC. Basophil CD203c levels are increased at baseline and can be used to monitor omalizumab treatment in subjects with nut allergy. *Int Arch Allergy Immunol*. 2011;154(4):318-27.