

Correspondence

Mast cell activation test versus basophil activation test and related competing issues

To the Editor:

First, we express our warmest congratulations for the recent article by Bahri et al,¹ published in the August issue of the *Journal of Allergy and Clinical Immunology*, in which the authors developed a robust and reproducible mast cell–based assay to improve the diagnosis of IgE-mediated allergy.¹ Our group is long since supporting the idea that circulating basophils in allergy are fundamentally regulatory rather than simple effector cells and hence mast cells should appear much more indicated as a suitable cellular model to diagnose allergy, due to their recognized effector function.² The new approach reported by the authors, called mast cell activation test (MAT), certainly antagonist to the cognate basophil activation test (BAT), uses human mast cells (hMCs) derived from c-kit^{pos} (CD117^{pos}), CD34^{pos} myeloblast precursors from bone marrow in the peripheral blood, then cultivated in buffered, cytokine supplemented media, and further sensitized with allergic patients' sera.¹ The approach is more time-consuming and somehow more cumbersome than BAT but mast cells have the advantage to be much more sensitive than basophils to allergen-induced degranulation and membrane CD63 upregulation.¹ The authors sensitized *in vitro* hMCs with sera from patients having either peanut or pollen allergy, then investigated the activation of hMCs in flow cytometry, and compared their performance with a commonly used BAT, showing a higher reliability, as the expression of CD63 in MAT needs a concentration of peanut allergens 2 log lower than in BAT.¹ Despite some difficulty in handling and standardizing the protocol for the routine diagnostic activity, the assay appears promising but we would like to address in this letter some major concerns.

Actually, hMCs are characterized by a huge heterogeneity and express their function as differentially shaped, according to the various microenvironments, where they are committed from bone marrow precursors.³ This might suggest that the generic source from blood precursors is inappropriate to achieve a reliable MAT, because of the existence of different allergic manifestations in different subjects. Other than tryptase or chymase-specific hMCs or both, a widest population of different functionally addressed mast cells has been reported, causing possible concern to the laboratory introduction of a MAT, which in this sense might be particularly questionable.^{3,4} Major differences in the function of hMCs should depend on signals coming from local tissues and from a certain memory with which these cells are endowed when challenged with allergens and immune stimuli.³ The functional heterogeneity of mast cells should be interpreted also in light of genetic polymorphism, which characterizes some genotypes of hMCs genes. For example, the existence of a chymase promoter gene polymorphism (CMA1, eg 1903G/A) might not make MAT a good tool to diagnose asthma susceptibility and/or severity, whereas it could correlate with serum IgE levels in eczema.⁴ Conversely, a single nucleotide polymorphism allele with a (TG)(n)(GA)(m) repeat polymorphism, 254 bp downstream of the CMA1 gene, has been significantly associated with asthma, whereas the CMA1 promoter polymorphism rs1800875 has been associated with atopic eczema but not with

serum IgE levels.⁵ Changes in the expression of mast cells chymase may affect the expression of c-kit, that is, of CD117, which is a phenotyping marker used in the described MAT.^{1,6}

Furthermore, hMCs are also somehow considered regulatory cells in allergy, an evidence that may garble our comprehension of hMCs, also for their functional heterogeneity.³ At least in BALB/c mice, food allergy may be exacerbated by intestinal mastocytosis, which could be even inhibited by plant-derived compounds.⁷ Food allergy–induced gut mastocytosis should represent a concern for the general use of MAT, particularly because cells express a very low or quite undetectable level of CD117 during mastocytosis.⁸ In this perspective, the CD117^{pos} mast cells, captured and tested in a MAT, may not be actually representative of the patient's allergic diagnostic panel and may even lead to a biased therapeutic approach. For example, Hymenoptera species sting allergy is particularly problematic in patients with mastocytosis.⁹

Moreover, Bahri et al used a novel approach for BAT in their study, despite the existence of a wide amount of previously assessed methods, as they used a CD203c phenotyping protocol to capture basophils endowed with negative gating markers, able to exclude CD3^{pos} lymphocytes and CD303^{pos} plasmacytoid dendritic cells.¹ Using a double-blind, placebo-controlled food challenge, the authors assessed that MAT showed the highest discrimination performance (area under the curve [AUC] = 0.99) compared with the indicated BAT (AUC = 0.94) and further diagnostic tests.¹ However, this potential was not adjusted on the higher CD63 membrane expression usually exhibited by peripheral mast cells in respect to circulating basophils. The authors reported that optimal cutoff values for CD63% were 7.8 and 10.5, respectively, for the whole population of subjects with peanut allergy (n = 42) and the cohort with equivocal skin prick test/serum IgE data (N = 24), versus a MAT, which gave an AUC of 6.3 for both conditions.¹ The comparison of MAT versus BAT is intriguing but a better suggestion would be the use of a commonly adopted BAT, while the phenotyping and gating approach of BAT adopted by the authors is rather unusual, in the current literature.

Our major concern is that the interesting approach reported by Bahri et al is particularly time-consuming and may fail in addressing a reliable diagnostic tool, fundamentally because of the cumbersome burden of the many puzzling issues related to heterogeneity of hMCs.

Salvaore Chirumbolo, PhD^a

Geir Bjørklund, MD^b

Antonio Vella, PhD^c

From ^athe Department of Neuroscience, Biomedicine and Movement Sciences, University of Verona, Verona, Italy; ^bthe Council for Nutritional and Environmental Medicine, Mo i Rana, Norway; and ^cAQUI Policlinico GB Rossi-Section of Immunology, Verona, Italy. E-mail: salvatore.chirumbolo@univr.it.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Bahri R, Custovic A, Korosec P, Tsoumani M, Barron M, Wu J, et al. Mast cell activation test in the diagnosis of allergic disease and anaphylaxis. *J Allergy Clin Immunol* 2018;142:485-96.
2. Chirumbolo S, Bjørklund G, Sboarina A, Vella A. The role of basophils as innate immune regulatory cells in allergy and immunotherapy. *Hum Vaccin Immunother* 2018;14:815-31.

3. Frossi B, Mion F, Sibilano R, Danelli L, Pucillo CEM. Is it time for a new classification of mast cells? What do we know about mast cell heterogeneity? *Immunol Rev* 2018;282:35-46.
4. Iwanaga T, McEuen A, Walls AF, Clough JB, Keith TP, Rorke S, et al. Polymorphism of the mast cell chymase gene (CMA1) promoter region: lack of association with asthma but association with serum total immunoglobulin E levels in adult atopic dermatitis. *Clin Exp Allergy* 2004;34:1037-42.
5. Sharma S, Rajan UM, Kumar A, Soni A, Ghosh B. A novel (TG)n(GA)m repeat polymorphism 254 bp downstream of the mast cell chymase (CMA1) gene is associated with atopic asthma and total serum IgE levels. *J Hum Genet* 2005;50:276-82.
6. Hermes B, Feldmann-Böddeker I, Welker P, Algermissen B, Steckelings MU, Grabbe J, et al. Altered expression of mast cell chymase and tryptase and of c-Kit in human cutaneous scar tissue. *J Invest Dermatol* 2000;114:51-5.
7. Kinney SR, Carlson L, Ser-Dolansky J, Thompson C, Shah S, Gambrah A, et al. Curcumin ingestion inhibits mastocytosis and suppresses intestinal anaphylaxis in a murine model of food allergy. *PLoS One* 2015;10:e0132467.
8. Escribano L, Diaz-Agustin B, López A, Núñez López R, García-Montero A, Almeida J, et al. Spanish Network on Mastocytosis (REMA). Immunophenotypic analysis of mast cells in mastocytosis: when and how to do it. Proposals of the Spanish Network on Mastocytosis (REMA). *Cytometry B Clin Cytom* 2004;58:1-8.
9. Bonadonna P, Zanotti R, Müller U. Mastocytosis and insect venom allergy. *Curr Opin Allergy Clin Immunol* 2010;10:347-53.

<https://doi.org/10.1016/j.jaci.2018.06.020>