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LETTER TO THE EDITOR

CD164 and other recently discovered activation markers as promising tools for allergy diagnosis: what's new?

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Dear Editor,

Patients who suffer from seasonal allergic rhinitis caused by allergy to grass pollen have become an important subject of investigation in recent years, as these patients, as like as asthmatics, are allergic but in primary care few diagnostic decisions and management interventions are made with formal allergy assessment [1, 2]. Moreover, there are few data on asthma risk factors and phenotypes in patients with seasonal allergic rhinitis, as severe rhinitis (together with poly-sensitization in adults) appears to be the dominant asthma phenotype [3]. In this context, classical skin test in allergic rhinitis is still considered a good diagnostic tool [4, 5], but in recent years, the evaluation of allergen-induced basophil activation by following surface marker up-regulation has become the hallmark of several reports about pollen allergy diagnosis [6–10].

A debate has opened about the sensitivity and specificity of the up-regulation testing in human basophils following challenge with a specific allergen, as the expression of basophil activation markers such as CD63 and CD203c detected by flow cytometry has attracted ongoing attention in allergy research field [11, 12]. More recently, in order to find a suitable tool to achieve a good diagnosis of allergy for immunotherapy and clinical management, many other membrane molecules able to highlight basophil response to external stimuli have been added to the list of potential markers [13]. Actually, a correct allergy diagnosis requires laboratory support to prevent potentially adverse systemic reactions during traditional clinical testing. The recent introduction of CD164 is a clear example of this trend [10–13]. CD164 is a sialomucin present in CD34-expressing cells, which is involved in cellular adhesion [14, 15] and plays an important role in haematopoiesis by allowing human CD34+ cells to adhere to stroma [10]. In basophils, this molecule is a member of the "fast responders", having been functionally associated with CD13 and CD203c [13] but, as for the ectoenzyme CD203c, the role of CD164 in allergy is currently unknown. Some authors have suggested that its expression in human basophil should follow the typical behaviour of CD203c. Wolanczyk-Medrala and colleagues reported that CD164 presumably reaches its maximal expression within 20 min from the stimulation, a value that is quite similar to previously reported data for CD203c, although CD164 expression has a smooth time course and CD203c reaches more than 60% expression within the first 5 min [10, 13, 16]. However, while CD203c appears to be solely expressed on basophils among blood cells, CD164 is expressed in other haematopoietic cells [17]. A role for CD164 reactivity is played by the differential epitopes expressed on basophils, as class III antibodies (67D2 and N6B6) show better performance in differentiating resting from activated basophils; so, a critical point for this marker when compared with CD203c might be the different expression on resting and activated basophils of different epitopes recognized by the antibody, an issue that might render more complex the analytical performance and product specificity of the latter [13]. When used as the specific activation marker in basophils gated with anti-CCR3 antibodies [18], CD164 shows a very high sensitivity and specificity at the highest allergen concentration used and a very good correlation with 0.2 μg/ml anti-IgE stimulation [10]. This high sensitivity is encouraging if one considers that it occurs without the use of IL-3 [11]. Taking into account that most of basophil

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activation tests (BATs), which evaluate CD203c for allergy diagnosis, usually include this cytokine as a priming agent, CD164 would appear widely more sensitive than CD203c, although this sensitivity drops down to 70% at allergen doses around 1.0 ng/ml, reaching values close to that performed by CD203c [12]. Nevertheless, to the best of my knowledge, no report comparing the analytical performance of CD203c and CD164 in an allergic model has been ever addressed, except few related considerations [10].

Performance evaluation of a defined analytical marker can be addressed by measuring sensitivity and specificity with known allergen concentration, and some authors have generated Receiver Operating Characteristics (ROC) analysis even for basophil activation markers: in the case of CD63 and CD203c, ROC curves were almost identical, leading authors to suggest that these two markers measure similar aspects of basophil activation [19]. So, it should appear that these two markers are good enough for any diagnostic use of basophil activation test (BAT) in allergy.

Notwithstanding, besides the interesting debate about the proper activation protocol and markers to be used in cellular allergy diagnosis, questions have been raised about the best gating procedure to isolate basophils from other cells in flow cytometry, and this have raised the need to search for new markers [20]. When a new activation marker is considered, it should be assessed also with the main gating strategies by which basophils are studied in flow cytometry. It should be very interesting, for example, to assess CD164 performance with other gating strategies, such as CRTH2/CD3 or CD123/HLA-DR [20]. A direct comparison of different methods and/or different BATs is, therefore, very difficult, due to the existence of several activation markers (CD63, CD203c, CD13, CD69, CD107a, CD164) and many gating approaches, besides intrinsic problems caused by basophil paucity in peripheral blood, heterogeneous responsiveness to external stimuli within normal population and allergen variability [12, 16, 20]. In this scenario, it is understandable why most of the physicians choose skin test and serum IgEs evaluation in primary allergy diagnosis and care. However, the reliability of these approaches is spoiled by many pitfalls, mainly due to discrepancies between IgE plasma levels and skin and/or cellular reaction [13].

The experience of many authors is, however, that clinical validation of various BATs in various ways for several years has shown that these new technologies have more potentials and perspectives than pitfalls [13]: this perspective should suggest that there are no serious reasons to deprive allergic patients of clinically indicated BAT, which can be performed reliably by any laboratory with allergy and flow-cytometric capacity and expertise. The application of flow cytometry in the study of basophil activation for the diagnosis of allergic diseases and asthma has given interesting results in recent years. The quantification of basophil activation by flow cytometry has been proven to be a useful tool for the assessment of the immediate-type response to allergens mediated by IgE or by other mechanisms such as drug hypersensitivity.

What is the challenge which lies ahead?

Routinely commercial basophil activation tests rely on the application of only two main activation markers, namely CD63 and CD203c; then, they do not seem to allow a forthright introduction of new molecules to follow basophil response to allergens or basophil biology in allergy. Basophils are yet very poorly considered, despite their increasing leading role in the immune response. Polychromatic flow cytometry (PFC) might achieve the purpose to gain new clues about the role of basophil in immunity but, maybe, it is not widely feasible as a diagnostic tool. One of the thorniest problems concerns PFC as a time-consuming test, yet an in vitro testing with blood may account for the same management cost of a lymphocyte subset analysis on a blood specimen. However, increased efforts should be directed not only at enhancing the performance of new diagnostic tools and new markers but also to highlight the role of the latter in describing basophil biology in allergy and inflammation. Of course, new markers can always be introduced in order to search for the better and less-expensive gating/activation protocol and to achieve the highest reliability and feasibility of basophil in vitro testing for allergy diagnosis.

It is desirable that deepening basic research of basophil through a more thorough investigation that also includes PFC may throw new light on allergy and ameliorate diagnostic tools state of art.

Conflict of interest The author reports no conflicts of interest in this work.

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