

CORRESPONDENCE

Clinical and Experimental Allergy

Is this time for CRTH2/DP2 in a flow cytometric basophil activation test?

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I have been particularly interested in the recent study of Abuaf et al. [1] comparing the use of two basophil activation markers (CD63 and CD203c) for the diagnosis of amoxicillin allergy. Since the year 1995, around 100 articles have been published regarding the diagnosis of allergy by a flow cytometric basophil activation test. This test (using either CD203c or CD63 as activation marker) has become a robust and reliable tool for *in vitro* investigations of immediate allergy [2]. There are mounting evidences that it is suitable for allergy diagnosis in clinical practice [2]. However, despite this quite abundant literature, few studies have been conducted so far comparing different protocols, although some technical issues remain to be addressed [3].

The present challenge is to establish a 'gold standard' protocol that can be standardized in multi-centre clinical studies. In that sense, the study of Abuaf et al. [1] represents a major contribution in the field as it provides two important results. First, their results, obtained in amoxicillin allergy, clearly indicate that the sensitivity obtained by using CD203c as an activation marker is higher than that of CD63 (60% vs. 20%, respectively – a result we previously reported in *Clinical and Experimental Allergy* in a context of latex allergy [4]). Second, they provide for the first time a clear demonstration that basophil recognition through the detection of surface IgE expression does not represent an appropriate method to ensure acceptable cell purity. Indeed, they observed a major contamination of basophils with monocytes (up to 50% of gated IgE⁺ cells). This drawback has been suspected for long (to explain some observed low sensitivities) but has never been appropriately investigated.

Furthermore, another drawback of this marker is that IgE expression on basophil surface is thought to be highly variable from one patient to another, rendering cell recognition (and thus recovery) difficult to standardize and in some cases virtually impossible. As an alternative and because of its selective expression on cells associated with T-helper type 2 (Th2) responses (Th2 lymphocytes, eosinophils and basophils), chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) has been proposed and validated as the most reliable tool for the detection of circulating human type 2 cells [5]. CRTH2 is also termed DP₂ because it is one of the two receptors of prostaglandin D₂ [6, 7]. As CRTH2 is highly expressed on basophils, we hypothesized that it may improve the basophil activation test by facilitating basophil recognition [8]. Consequently, we developed a three-colour protocol (CD203c/CRTH2/CD3) for monitoring allergen-induced basophil activation by flow cytometry. Briefly, on the basis of light scattering, eosinophils are first easily excluded from the analysis of CRTH2-expressing cells. Then basophils can readily be distinguished from Th2 lymphocytes based on CD3 expression. Finally, among this gated population of basophils, the modulation of CD203c after allergen challenge is monitored as described in Abuaf's manuscript. Preliminary results were excellent [8]. Since its discovery, no expression of CRTH2/DP2 on non-type 2 cells has ever been reported [9, 10]. This receptor is thus highly and specifically associated to allergic processes and appears as a very promising tool for the improvement of the basophil activation test by flow cytometry as it may solve the question of the gating strategy. Regarding the latter, the sole end-point is the basophil recovery and purity; we need to include and monitor all basophils (recovery) but we also need to exclude all non-basophil cells (purity). The only suitable approach to address this question is to perform cell sorting based on different gating strategies. In such experiments, I would highly suggest – from a flow cytometry unit supervisor's view – considering the assessment of CRTH2/DP2 expression.

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