

Basophil Activation Tests Based on CD193 Marker in Dipyrone Allergy

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Dear Editor,

I recently read the article *Threshold for Positivity and Optimal Dipyrone Concentration in Flow Cytometry-Assisted Basophil Activation Test* by Hagau *et al.*, in the August 2013 issue of AAIR, where the authors assessed the usefulness of basophil activation tests (BATs) in the diagnosis of allergy to dipyrone.¹ Allergy to dipyrone still represents a main concern and pyrazolones are considered a major cause of immediate IgE-mediated reactions to drugs in many countries.² A case of drug rash with eosinophilia and systemic symptomatology (DRESS) attributed to dipyrone was recently reported; however, the authors could not rule out a possible interfering role attributed to herbal mixtures and other drugs.³ BATs represent an interesting tool to diagnose drug hypersensitivity, though with some criticism about their performance and interpretation.⁴ Basophils captured in flow cytometry by CD193 (CCR3)- phycoerythrin (PE) may be contaminated by CD3⁺ expressing cells, leading to possible bias.⁵ Despite this issue, Hagau and colleagues reported that commercial BAT equipped with a Flow2-CAST technique, showed a sensitivity as low as 0.25 µg/mL dipyrone, about 3 orders of magnitude lower than previous reported evidence.^{1,2} In the paper by Gomez *et al.*, a commercial BAT using an anti-IgE/CD63 protocol (instead of a CCR3/CD63) reported that the lowest dose able to trigger CD63 upregulation over the 5% threshold, was 0.25 mg/mL dipyrone (matamizole); Hagau and co-workers used the same CD63% threshold.¹ It appears quite difficult to attribute this sensitivity improvement to superior BAT performance (at least in regards to the phenotyping protocol).⁵ CCR3 has been recently reviewed as a phenotyping marker and compared to other flow cytometry approaches.⁵ The eotaxin receptor is commonly expressed on eosinophils,⁶ together with CD63 (which is only present on activated cells)⁷; however, in a SSC (side scatter)/CCR3 scatter plot, eosinophils and basophils can be clearly separated and eosinophils should not affect a

Flow2-CAST BAT performance. Yet CCR3 is downregulated during basophil activation⁵ and the proportion of gated basophils evaluated as up-regulating CD63 is higher than the same calculated on resting, non activated basophils, if gate is set at a fixed threshold (usually ≤ 5%). In a SSC/CCR3 gating plot basophils may be underestimated, due to CCR3 downregulation and contamination by SSC^{low} scattered /CD3⁺ lymphocytes. If we consider that CCR3-PE^{bright} basophils express homogeneously very low amount of membrane CD63 (for example 5%), a supposed reduction of 52% of 500 gated CCR3-PE^{bright} cells (due to activation), might shift the threshold to 1.53%. This would mean that a proportion of CD63^{neg} cells enter the right side of the threshold and lead to a higher CD63% evaluation. The bias may be significant when CD63% is close to the threshold. In the paper,¹ SI is calculated on the number of basophils and Table 1¹ reports 6/20 samples (30%) with 5.3 < SI < 9.1. The simultaneous evaluation of CD63-FITC_{MFI} and CD63% should prevent a bias⁴; however, other bias can still occur. The operator might capture more CCR3-PE^{bright} cells than the negative control: in this case, as CCR3-PE^{bright} basophils are negative, the MFI ratio CD63^{pos}/CD63^{neg} may decrease while CD63% increase. In the lack of a CD3 marker, the operator may capture low SSC cells that express CCR3 but not CD63: this occurrence may also decrease the MFI ratio CD63^{neg}/CD63^{pos} and increase CD63%. Lowest doses of dipyrone may show a low expression of CD63-FITC (as median MFI) but relatively higher CD63% expressing basophils. This may prompt researchers to evaluate BAT as a

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valuable tool to probe very low doses of dipyrone in allergy; however, the absence of a flow cytometry plot reported by the authors¹ makes it difficult to address this point and to elucidate possible causes about the high sensitivity reported.

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