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Basophil Activation Tests Based on CD193 Marker in Dipyrone Allergy

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I recently read the article *Threshold for Positivity and Optimal Dipyrone Concentration in Flow Cytometry-Assisted Basophil Activation Test* by Hagau *et al.*,[1] 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. 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 apppears 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 activation[5] 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 1[1] reports 6/20 samples (30%) with 5.3< SI <9.1. The simultaneous evaluation of $CD63\text{-}FITC_{MFI}$ and CD63% should prevent a bias[4]; 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 valuable tool to probe very low doses of dipyrone in allergy;

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however, the absence of a flow cytometry plot reported by the authors[1] makes it difficult to address this point and to elucidate possible causes about the high sensitivity reported.

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