

## LETTER TO THE EDITOR

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# The CD45<sup>dim</sup>/CD123<sup>bright</sup>/HLADR<sup>neg</sup> BAT in the Anti-histamine Drug Allergy

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## To The Editor

A recent paper by Rial et al reported the suitable use of a basophil activation test (BAT) in the diagnosis of hypersensitivity to the first-generation antihistamine doxylamine.<sup>1</sup> They adopted a side scatter (SSC)/cluster of differentiation (CD) 203c, i.e. SSC<sup>low</sup>/CD203c<sup>pos</sup> phenotyping protocol to capture basophils in the blood sample of the pregnant woman and a CD63<sup>pos</sup> marker as the activation probe.<sup>1</sup> The results showed by Rial et al, although referring to a single case report, are evocative and clearly exhibit a marked difference in the pregnant woman sample's CD63% compared to the control sample.<sup>1</sup> Aside from the lack of reliable statistics, we recently expressed a certain criticism about the use of a SSC<sup>low</sup>/CD203c<sup>pos</sup> gating strategy, because we widely demonstrated that CD203c is upregulated during the cellular activation, causing bias in the CD63% evaluation.<sup>2,3</sup> In a recent evaluation in our laboratories (data from 2009 to 2011), a marked difference in the CD63% have been shown if adopting an SSC<sup>low</sup>/CD203c<sup>pos</sup> BAT (grey box plots) respect to a CD45<sup>dim</sup>/CD123<sup>bright</sup>/HLADR<sup>neg</sup> BAT (white box plots) (Figure 1). Although the difference in percentage does not appear so striking yet giving a significant drift in the evaluation of the CD63%, analyzing basophil with a phenotyping method respect to another one can result in possible opposite diagnostic decisions, depending on

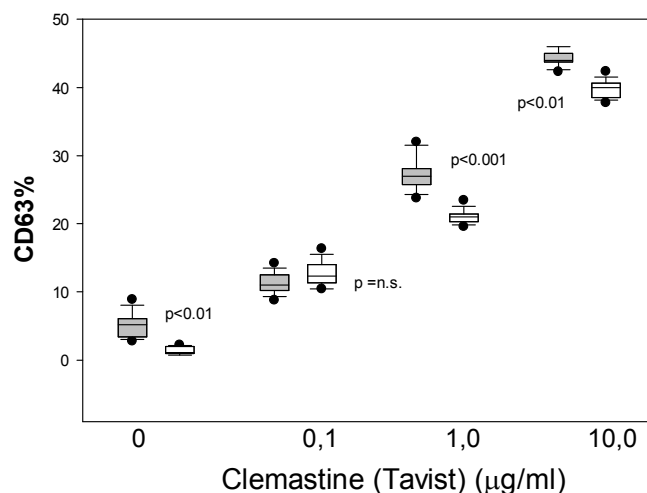
a phenotyping method respect to another one can result a certain CD63 threshold.<sup>2,4,5</sup> Furthermore, a case report in our past studies, male 52 yrs old, with a previous diagnosis of an anaphylactoid reaction to Tavist six months before the test, underwent an SSC<sup>low</sup>/CD203c<sup>pos</sup> BAT (BasoflowExVR kit; Exbio, Praha, Czech Republic), to verify if his current skin rashes, itching, and hives, with a moderate and apparently exacerbating with time lips swelling, might be due to the intake of Tavist (clemastine fumarate, Novartis Consumer Health Ltd, USA), tablets, about one-two hours before. The patient interrupted this therapy and underwent treatment with 30 mg per oral suspension (os)/day of diphenhydramine (Benadryl, Johnson & Johnson, NJ, USA), with a poor outcome. The patient, with a BMI $\geq$ 30 (34.19), had an anamnestic history of allergic rhinitis and developed idiopathic urticaria in closest weeks before his recruitment, for which he undertook 2.68 mg clemastine per os any 8 h for two days. On day three after treatment, he suffers from the chest and abdominal pain and was hospitalized for angioedema and pulmonary edema. The diagnosis was performed by specialized physicians of the University Hospital, Unit of Respiratory Physiopathology, who took also into account the previous diagnosis addressed by his dermatologist, whom the patient referred to at days 1-2 before hospitalization. Anaphylactoid reaction was assessed by the occurrence of moderate hypotension, complement activation and cutaneous eruption.<sup>6</sup> Serum IgE was negative (25.87 UI/mL, threshold $\geq$ 100 UI/mL) and tryptase test positive ( $\geq$ 15  $\mu$ g/L).<sup>7</sup> The BasoflowExVR gave a CD63% of about 55.65% $\pm$ 12.44 SD in a triplicate sample. This occurs for three separate

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runs, while using an in-home protocol capturing basophils as  $CD45^{dim}/CD123b^{right}/HLADR^{neg}$  the CD63% was  $21.44 \pm 2.25$  SD, a result that allowed clinicians to exclude a forthright anaphylactic episode and to assess an urticaria reaction. They were

encouraged to deepen further allergic data regarding the case report. Moreover, CD63% thresholds (cut off) for the gating A (BasoflowExVR kit) was  $\leq 15\%$ , for the gating B ( $CD45^{dim}/CD123^{bright}/HLADR^{neg}$  BAT) was  $\leq 5\%$ .



**Figure 1.** Box Whisker plots of 15 samples of patients and 14 healthy donors coming in our caregiving structures undergoing a BasoflowExVR kit (grey) or a  $CD45^{dim}/CD123b^{right}/HLADR^{neg}$  test (white) (BD FacsCanto II, dates sept 2009-April 2011) for the allergy to the first generation anti-histamine drug clemastine. Patients suffered from hypersensitivity type I response with urticaria-like reaction with itches and mouth swelling (7 cases), asthmatic-like symptoms with bronchospasms (5 cases), 1 anaphylaxis, 2 chronic rhinitis, and cutaneous eczemas, after being treated with Tavegil tablets (1 case), Tavist tablets (10 cases), Tavist Syrup (4 cases) ((Novartis Consumer Health Ltd), at least one week before the BAT performance. Patients distribution was accounted as having a mean age  $54 \pm 3.54$  SD (female/male ratio 0.65), controls  $56 \pm 5.87$  SD, female/male ratio 0.47). BATs were performed by challenging basophils with clemastine fumarate (Sigma Aldrich, USA, CAS 15686-51-8)) at the dosage ranges used in treatments. Statistics were performed with a Wilcoxon rank test ( $p < 0.05$ ) (SPSS v 24.0). The study underlining this plot was performed to demonstrate the different BAT behaviors to clemastine fumarate-induced hypersensitivity, according to the different gating protocols. BATs were carried out at the BD FacsCanto II™. Monoclonal antibodies conjugated with fluorochromes were purchased from BD Pharmigen, Germany.

Independent research investigation allowed us to calculate that the use of a  $SSC^{low}/CD203c^{pos}$  gating strategy, due to the gating shift to which the membrane marker CD203c is subjected upon cell activation, caused a loss in the gated events captured as  $CD203c^{pos}$  cells (i.e. basophils) from 7 to 36%.<sup>2,3</sup> This should lead to a number of gated events (basophils) expressing CD63 sensitively lower than the events at the CD63 baseline, causing a bias in the final evaluation of CD63%.

Although the paper by Rial et al is highly suggestive of the allergic role of a first generation anti-histamine, such as doxylamine, more accurate analysis

of the basophil capturing in flow cytometry may help clinicians in doing better their diagnostic decision.

#### Conflict of Interest

The Authors state they have no conflicts of interest.

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## A New BAT Protocol for Anti-histamine Drug Allergy

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