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Short communication

Bias in the use of a $SSC^{low}/CCR3^{pos}$ gate to capture basophils in chronic urticaria?

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ABSTRACT

A comment on D'Auria E., De Amici M., Licari A., Caimmi S., Mantegazza C., Zuccotti G. and Marseglia G., Basophil activation test in children with autoimmune chronic spontaneous urticaria: Iis it ready for clinical practice?, Immunobiology 224 (1), 2019, 30–33.

To the Editor,

In a very recent paper on this journal, a pilot study from D'Auria et al., suggested the use of the basophil activation test (BAT) as a diagnostic tool able to identify chronic urticaria (CU) subtypes in children (D'Auria et al., 2019). The authors adopted a commercial kit (FlowCAST®, BUHLMANN Laboratories, Schonenbuch, Switzerland), where basophils from peripheral blood samples are electronically captured as $SSC^{low}/CCR3^{pos}$events in a flow cytometry (FC) approach. The use of the phenotyping marker CCR3 in BAT has been widely criticized, due to its co-expression in Th2 cells, which co-localize with basophils in the SSC^{low} gate (Monneret, 2010). The possibility that $CCR3^{pos}$ T cells are included in the SSC^{low} gated basophils cannot be prevented, unless a CD3 marker is considered in order to remove from the $CCR3^{pos}$ population any notoriously $CD3^{pos}$ T cell (Monneret, 2010). D'Auria et al., concluded that BAT allowed to discriminate about 37.5% of children with autoimmune urticaria (AIU), a value appearing to confirm previous reported data (Sahiner et al., 2011; Konstantinou et al., 2013).

The purpose of this Letter to the Editor is to comprehend some issues that generated doubts and criticism in our group, while reading the paper, hoping to expand the debate with the authors and further colleagues.

The CD63% cut off reported by the authors is $\leq 5.0\%$, therefore, standing on this threshold, only six patients showed values higher than 5%. In this perspective, the authors appear to be right in concluding a percentage of 6/16 = 37.5% (D'Auria et al., 2019). However, the authors reported also, following the BAT producer's instructions, that positive values were calculated on a stimulation index (SI), i.e. the ratio between $CD63^{pos}$ % basophils from AIU and those ones from controls, which must result ≥ 2.0. If true, positive values dropped from 6 to 1 single subject (CD63% = 14.58, age 10 yrs), who interestingly showed ANA pos 1:80 and serum IgE for inhalant allergens (pollens from birch and grasses) (D'Auria et al., 2019). Spearman correlation between CD63% and serum IgEs performed from ours, was not significant (rho = −0.0757, p = 0.50) and this lacking of correlation can be observed also for positive CD63% (rho = +0.5822, p = 0.10). This confirms some previously reported articles about the lack in correlation between serum IgEs and CD63 expression in basophils of patients with urticaria, where the positive CD63% subjects with ASST positive test were 31.6% (Curto-Barredo et al., 2016; Sterba et al., 2015).

The phenotyping protocol may affect dramatically BAT performance, particularly in the CD63% evaluation (Chirumbolo, 2014). While performing a BAT gated as $CCR3^{pos}$ cells, we can introduce also T cells expressing the tetraspanin CD63 (Pfistershammer et al., 2004; Petersen et al., 2011). CD63, i.e. the lysosomal-associated membrane protein-3 (LAMP-3), is poorly expressed in $CCR3^{pos}$ resting T cells but, likewise basophils, the marker is rapidly shifted in lymphocytes from the intracellular lysosome-associated granules to the membrane upon stimulation (Pfistershammer et al., 2004). During chronic idiopathic and autoimmune urticaria $CD3^{pos}$ $CD4^{pos}$ Th cells are directly implicated in the pathology, including a down-regulation of the FcεRI and related allergic activation in basophils of urticaria subjects (Caproni

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et al., 2005). As $CCR3^{pos}$ cells, Th cells can contaminate the SSC^{low}/$CCR3^{pos}$ gate, used to capture basophils in FC, without the introduction of a CD3 marker. The possibility of bias from introducing further $CCR3^{pos}$ cells in the purported basophil gate, particularly if also expressing CD63, is a real concern for BAT suitability in autoimmune urticaria (AIU) or other forms of chronic urticaria. Further BAT protocols were reported in subjects with urticaria, for example the gating protocol CD123-PE/anti-HLA-DR-PerCP (Chen et al., 2017; Chirumbolo, 2012); in this circumstance the positive CD63% urticaria subjects were about 69.8%, of which 39.68% with allergy, a value interestingly close to the reported 37.5% from D'Auria et al., although the actual value for positive CD63% results with allergy was 6.25% (D'Auria et al., 2019; Chen et al., 2017). The quite equivocal data of positive CD63%, expressed as events $CD63^{pos}$ in the $CCR3^{pos}$ gate cannot exclude the introduction of more CD63 expressing cells other than basophils in the gate.

A possible criticism to this hypothesis may be an expected increase in CD63% in the SSC^{low}/$CCR3^{pos}$ gate, due to the contamination of actively CD63 expressing Th cells. We suppose that neither authors' evidence nor our debating opinions are able to warrant for the exclusive evaluation of basophils CD63, whereas lacking a CD3 marker in the BAT.

Despite these considerations, the use of an alternative BAT protocol, where the cross contamination with T cells is prevented, confirmed that basophils from individuals with urticaria increased membrane CD63 up-regulation (Chen et al., 2017).

In this sense, BAT can be suggested as a reliable and useful tool in chronic urticaria, paying attention to the numerous pitfalls and bias hidden in a routinely performed analysis.

Transparency document

The Transparency document associated with this article can be found in the online version.

Conflict of interest

The authors state they have no conflict of interest.

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