

## Correspondence

**Expression of activation markers on basophils in a controlled model of anaphylaxis: General, methodologic, and clinical issues**

To the Editor:

We appreciated the article by Gober et al<sup>1</sup> considering flow-assisted analysis to monitor the effects of intentional stinging challenges. However, we would like to address several issues.

First, the authors state that CD63 is rapidly mobilized on the basophil surface by IL-3. In fact, priming with IL-3 does not elicit upregulation of CD63 expression by itself.<sup>2,3</sup> Remarkably, both of these articles are referred to in this article. The Yoshimura article<sup>2</sup> is thus wrongly cited. For years, we used flow cytometry in the diagnosis of Hymenoptera venom allergy and follow-up of venom immunotherapy (VIT). Although our analyses are restricted to patients with systemic reactions (large local reactions not being an indication for VIT<sup>4,5</sup>) and we have been using a whole blood assay, we were surprised by some findings here. In our experience insufficient basophil recovery is rare. Therefore we are surprised by a drop-out of 14 of 35 patients. Although little information is provided about the basophil isolation technique, it is somewhat surprising that only a purity of 8% was reached. Others,<sup>6</sup> using an almost similar technique, report a basophil purity of greater than 80%. It is well known that improper isolation techniques could result in *in vitro* activation of basophils having higher basal CD63 expression. Having performed lots of basophil activation tests in patients before and at different time points during early and prolonged VIT, we did not observe increased spontaneous expression of CD63 compared with that seen in asymptomatic control individuals.<sup>7</sup> In other words, our data do not support the hypothesis of a primed phenotype in patients with venom allergy.

The authors state that the allergen doses applied in basophil activation tests rest on experience with mediator release tests. They suggest that basophil activation tests could benefit from concentrations more relevant to those achieved after *in vivo* stings. However, the authors failed to identify some relevant publications. Several studies on flow-assisted venom allergy diagnosis with dose-finding experiments between patients and control subjects have been published.<sup>7-9</sup> One should also be aware that the published stimulation concentrations are “working concentrations” and frequently do not reflect the final concentrations in the aliquots.

Finally, the authors state that allergen sensing by basophils, as demonstrated by CD63 expression, persists

despite VIT. However, in the cited article,<sup>9</sup> according to their dose-finding experiments, the authors applied maximal stimulation of the basophils before stinging challenge. We demonstrated venom-induced CD63 upregulation to significantly decrease during VIT, provided the cells are stimulated submaximally.<sup>7</sup> The final concentration of venom in the aliquots to demonstrate this effect was 0.005 µg/mL, a concentration considered to be clinically relevant.<sup>1</sup>

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