

Letter to the Editor

The Use of IL-3 in Basophil Activation Tests is the Real Pitfall

I have recently read the article by Sturm et al. in the latest issue of *Cytometry B Clinical Cytometry* (1), to which I agree with many of the Author's conclusions, though I have some considerations to address.

The article tries to assess the use of basophil activation tests (BATs) based on CD203c upregulation as a reliable tool for allergy diagnosis. BATs, based either on the increased expression of CD63 or CD203c by activated basophils, have been proposed in many issues as a promising diagnostic tool for drug hypersensitivity, hymenoptera venom allergy, or other allergic pathologies (2); criticism has raised the question whether baso-tests are potential good analytical approaches or pitfalls (3). CD203c is rapidly upregulated as other less known activation markers (CD164 and CD13) and maximal ectoenzyme updisplacement on basophil membrane are reached after 5–15 min following stimulation (4,5). Both the granule-associated tetraspan CD63 and the ectoenzyme CD203c are upregulated to the plasma membrane of basophilic cells in response to FcεRI crosslinking, which can be triggered both by allergens and anti-IgE immunoglobulins. Upregulation time-course of these markers seems to be rather tricky to evaluate the quality and the amount of basophil activation response to an IgE-mediated triggering agent. In the work of Sturm and colleagues, CD63 and CD203c upregulation are affected both by activation time and by prestoring blood samples at room temperature at different times (1). This observation raises the question whether it is possible to standardize a basophil resting (nonactivated) condition and to evaluate the amount of expression of membrane markers with a reference standardized evaluation, which could be used in any circumstance: in this context, critical factors are represented by basophil spontaneously induced activation,

by intracellular calcium and the presence of calcium chelators in the medium, and by time and temperature. However, the proposal is hampered by the wide variability of basophil response to allergens, due to different levels of non-releaser and releaser subjects, by seasonal factors and by experimental conditions (3), and we are possibly in a bottleneck quite similar to that of coagulation international normalized ratio values. Blood microenvironment can preactivate leukocytes by releasing several priming factors but also basophils can produce IL-3 within 4 h in response to IgE-dependent activation (6). Within 4 h, IL-3 production reaches its peak but its release begins in the first hour following peripheral blood withdrawal (6). Hence, autocrine IL-3 release by basophils in blood stressed by venipuncture and vial microenvironment is reported to be the most critical point of preanalytical factors able to influence BAT diagnostic performance. A low level of anti-IgE/FcεRI crosslinking triggers IL-3 autocrine production to prime basophils to respond, so even anti-IgE concentration might be a critical point (6). Basophils rapidly bind and use the IL-3 they produce but this effect is inhibited in the presence of neutralizing anti-IL-3-α receptor, namely CD123 (6): this could raise the question whether incubation of anti-CD123-fluorochrome labeled antibodies and exogenous IL-3 in the same cellular environment could be optimized. One way to carry out experiments with resting basophils keeping a baseline level until their stimulation is to use ice bath. I wonder whether basophils could actually be primed by autocrine IL-3 in the 4 h following venous blood withdrawal, especially if receptor-ligand function is blocked by treating basophils in ice bath: a spontaneously induced priming by autocrine IL-3 should justify the conclusion with which Sturm et al. suggest how to

use BATs that evaluate CD203c upregulation. Hence, one issue is either to start experimental condition very early or by using ice bath for treating basophils, even during staining with fluorochrome-labeled antibodies (5). Factors other than IgE-mediated agonists, such as anaphylotoxins or bacterial products, are not able to induce autocrine IL-3 by nonstimulated basophils; hence, preanalytical errors due to environmental contamination are not relevant for basophil evaluation, whereas what seems to be more critical is the role of intracellular calcium (6). The latter condition along with a relatively high temperature could be the main factor affecting basophil spontaneously induced autocrine priming; on the other hand, calcium chelators such as ethylene-diamine-tetraacetic-acid (EDTA) or ethylene-glycol-tetraacetic-acid (EGTA) in blood microenvironment might affect activation testing (3). Another issue is that basophils may very well regulate their own priming, namely that the priming mechanism involves a fine regulation of IL-3 receptor recycling and desensitization/downregulation to further IL-3 providing. This makes crucial the use IL-3 in the long-standing idea that in vitro basophil releasability is clinically relevant (7). Basophils from allergic patients can be primed compared with those of non-allergic subjects; in the latter case, priming effect induced by exogenous IL-3 of the former might lead to underestimate CD203c upregulation following anti-IgE stimulation, mainly when it is compared with exogenous IL-3 primed nonallergic samples. The same reasoning could be forwarded by considering IL-3 spontaneously induced priming by preanalytical condition. We are unable to know if by treating basophils with exogenous IL-3, IgE-mediated response of CD203c is downregulated or not.

Therefore, besides to the preanalytical and experimental factors here

reported, inducing IL-3 priming in BATs to increase test sensitivity is the real pit-fall.

CD63 is a marker, which is upregulated within the first minutes following stimulation (5); its upregulation is rapid and marked, and it does not need for priming basophils. As IL-3 is a critical point for constitutively expressed markers, such as CD203c, a reappraisal about the use of IL-3 in BATs should definitively be addressed.

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