

Letter to the Editor

Opioid Non-IgE Mediated Effects in a BAT Assay on Drug Hypersensitivity to Pholcodine

Dear Editor,

I recently read the article by Leysen and colleagues in which pholcodine, an opioid compound used as a cough suppressant, has been associated with allergic reaction (1). The challenge of *in vitro* basophils with 10 µg/ml pholcodine resulted in 29% CD63 positive cells with completed release of their histamine content, by using an intracellular diamino oxidase test (2). Furthermore, about 43% of basophils challenged with pholcodine did not exhibit neither CD63 up-regulation, nor histamine release, while only 24% released histamine apparently without expressing CD63 onto the membrane (1). On the contrary, the anti-IgE positive control elicited a very low percentage of CD63 positive histamine releasing basophils (11%, Figure 2 in the paper) and, if considering that 5% is probably due to spontaneously activated basophils, this value should be around 14%, very close to the neg/pos CD63% threshold (10%) (1). Actually, a number of basophil activation tests (BATs) suffer from a certain discrepancy between anti-IgE positive control and test results, a fact that may come from methodological setting and basophil response to the agonist or allergen (3). Probably, anti-IgE as a tracking molecule to capture basophils should be reviewed. The Authors used an anti-IgE-^{pos}/CD203c^{pos} phenotyping protocol to capture basophils from heparinized whole blood; apparently, this seems a good approach to gate cells selectively but both anti-IgE and CD203c are closely related to basophils in the activated state, that is, anti-IgE may activate basophils and CD203c modifies its expression on cell membrane following activation. This fact makes the markers less reliable than others known to be not influenced by the activation process (4). Actually, CD203c is a selective marker for basophil phenotyping, but some comments were recently raised about its reliability in flow cytometry

protocol (3). The evaluation of % activated basophils may be perturbed therefore by preanalytical errors (4). Basophils preactivated by phenotyping anti-IgE, may down regulate surface FcεRI so resulting in a poor anti-IgE response. Notwithstanding, in an IgE-mediated allergy, the rate of CD63 up-displacement on basophil membrane following the allergen challenge should be comparable to that of an anti-IgE positive control. If this does not occur and the positive control in a BAT works weakly, a possibility to explain a significant CD63% value by using the indicated allergen, is a non-IgE mediated response to the molecule. Past reports have shown the existence of opioid receptors on immune cells (5,6) and opioid-induced modification of granulocyte activity (7). Pholcodine may activate immune cells contained in blood samples causing a modulation in intracellular signaling (8). A non-IgE dependent stimulus may induce a loss of Syk in human basophils (9), then a reduction in IgE-mediated response: this evidence may give further suggestion about the low responsiveness observed to the anti-IgE control.

The discrepancy between serum and cellular test is another hallmark of BAT-related methodological concerns and, at least in this case, may assess the speculation about a non-IgE mediated allergy. In the paper (1), CD63% membrane up-regulation occurred with very low doses of pholcodine in patient (1), which presented the highest total sIgE level (6,500 kUa/L), but relatively low doses of anti-pholcodine specific serum IgEs. Conversely, both patients 1 and 2 indicated in Table 1 of the manuscript (1), had a significant serum level of antimorphine specific IgEs. The increase in the dose-response activity of CD63 membrane up-regulation related to pholcodine was closely associated with total serum IgE levels, but not with specific antipholcodine IgEs. The paper reported that antipholcodine serum IgEs were lower than morphine-related sIgEs (1). This discrepancy

between IgE serology and BAT, if considering the allergic presumptive symptoms of the recruited patients, should ask for an optimization of BAT performance as a more reliable assay to diagnose drug hypersensitivity in those subjects who underwent a drug treatment.

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Salvatore Chirumbolo

Department of Medicine

University of Verona

LURM-Est Policlinico GB Rossi

Piazzale AL Scurio 10

Verona 37134, Italy

E-mail: salvatore.chirumbolo@univr.it

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