

Use of basophil sensitivity not reactivity as a good marker for allergy diagnosis

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Introduction

Human basophil activation has been analysed by several methods, mainly histamine release, leukotriene release and flow cytometry. Regardless of the readout, an "activation curve" (bell shape or sigmoid shape) is obtained by testing at least three allergen dilutions simultaneously. The optimal activating dilution is variable from one allergen to another. However, for the same allergen, the activation range is relatively stable over one or 2 logs of concentrations (personal observation).

Human basophil reactivity may be modulated by different factors, inhibitors such as IgG and IgA immunocapture, activation of ITIM motifs by IgE/IgG/allergen complexes, down regulation of IgE receptors and activators such as the presence of "priming cytokines (IL3, IL5), up regulation of IgE receptors and increase of the specific IgE/total IgE ratio. For a given immunological status, basophil reactivity may also depend on the affinity of specific IgE for the allergen, the allergen concentration, the inter-epitope distance, contact delay between the allergen and the related membrane receptor bound specific IgE and the intrinsic basophil reactivity. In order for human basophils to become activated a number of conditions must be met: allergens must be at least bivalent; at least 40 molecules IgE must be bound to their specific receptors per basophil; the optimal inter epitope distance must be between 8–24 nm; the allergen concentration cause a minimum of 100 cross links/cell; the minimum contact time is about 100 s and must result in a minimum of 0.26 % receptor clustering [1, 2].

The two main parameters given by these activation curves are the maximum response (reactivity) and the slope of the activation curve (sensitivity). The aim of this study was to test the hypothesis that sensitivity is a powerful tool for evaluating the allergological patient's

status than reactivity. We therefore tested several mathematical approximation methods and looked for an efficient way of expressing basophil sensitivity. D20, defined as the allergen concentration which causes 20 % CD63 expression, was chosen and we applied the calculation to venom allergy diagnosis and to the follow up of venom immunotherapy.

Material and methods

Patients were selected on the basis of the clinical history, skin tests (IDR) and specific IgEs.

Six patients had a clinical history of local/loco-regional reaction (group 1, without generalized reaction, bronchoconstriction or drop of blood pressure); seven patients presented with an acute/systemic reaction induced by a wasp sting (group 2) and 15 patients who had been treated for at least five years by specific immunotherapy (SIT, group 3) with wasp venom. This latter group of patients had initially presented with a systemic reaction with both positive skin tests and specific IgE.

Basophil activation induced by venom was analysed by flow cytometry. Blood was taken on EDTA and basophil suspensions were prepared by the buffy coat method already described [3]. Cells were resuspended in a calcium buffer (127 mM NaCl, 5 mM KCl, 20 mM TRIZMA, CaCl₂ 5mM, MgCl₂ 2 mM, 5 IU/ml heparin; pH 7.4). A series of wasp venom (Stallergenes laboratories, France) dilutions were prepared in the same buffer (1, 0.33, 0.11 µg/ml). Cell suspensions were mixed V/V with calcium buffer (negative control), anti-IgE (anti-D2 monoclonal, Immunotech, France) or venom dilutions. After 30 min incubation at 37°C, basophil activation was stopped by addition of 50 µl of EDTA buffer (127 mM NaCl, 5 mM KCl, 20 mM TRIZMA, EDTA Na₃ 2.8 mM, 5 IU/ml heparin; pH 7.4). Cells were then labelled with a mixture of anti-IgE FITC (Caltag, USA) and anti-CD63 PE (Immunotech, France). After 10 min in the dark, erythrocytes were lysed with an ammonium chloride buffer (NH₄Cl 154 mM, CO₂HK₂ 10 mM, EDTA Na₃ 0.08 mM, pH 7.2). After 10 min at room temperature, leukocyte suspensions were centrifuged (400 g, 5 min) and 500 µl of sheath buffer (Isoflow, Beckman Coulter, USA) added. Results are expressed in percentage CD63 IgE++ cells.

D20 was calculated by a second degree polynomial approximation (Excel software) and calculation of the roots of the related equation led to the venom concentration which caused 20 % CD63 expression (and not 20 % of the maximal response).

These experiments have been approved by the local ethics committee and patients have given their informed consent.

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Results and discussion

CD63 expression related to the 3 venom concentrations and to the 3 groups of patients was respectively: 1 µg/ml ($44.5 \pm 22.6\%$, $72.9 \pm 22.9\%$, $23.1 \pm 23.5\%$), 0.33 µg/ml ($10.1 \pm 6.5\%$, $66 \pm 24.3\%$, $1.6 \pm 1.9\%$) and 0.11 µg/ml ($1.8 \pm 1.7\%$, $37.6 \pm 35.6\%$, $2.9 \pm 3.5\%$). Although the concentrations 0.33 µg/ml and 0.1 µg/ml seemed to be discriminative there is a partial overlap of the results. Calculation of the D20 led to the following results: group 1 (0.6, 0.39, 0.34, 0.59, >1, 1), group 2 (0.08, 0.05, 0.04, 0.07, <0.04, 0.14, 0.21), group 3 (0.8, 0.8, 0.8, >1, >1, >1, >1, >1, 0.8, >1, >1, 0.7, >1, 1). D20 was chosen instead of the usual D50 due to the low percentage activation generally observed after SIT.

A clear cut gap for D20 values was observed between group 2 and groups 1 and 3 and set the threshold at 0.30 µg/ml before having a sufficient amount of data to calculate a more precise value. Sensitivity measured using the D20 for specific immunotherapy resulted in values in the range of the D20 observed for the local/loco-regional reactions.

Different approaches to assess human basophil sensitivity have already been published: D50 defined as 50 % of the maximal response (4–5), allergen threshold (6) and the number of cell-surface IgE molecules required to obtain a half-maximal IgE-mediated response (7). Interestingly, these different studies were in favour of the evaluation of basophil sensitivity which provided other information than the maximal response usually given as the only result of allergen induced basophil activation.

In particular, the use of the allergen threshold (6) led to a significant correlation between basophil response and skin prick tests, nasal provocation titre and even ratio specific IgEs/total IgEs whereas reactivity did not corre-

late with any of these parameters. The use of the measurement of the number of bound IgEs led to the conclusion that sensitivity and reactivity were independent variables. Among these different approaches, the D20 as defined here, independent of the maximal response may lead to a better inter individual differentiation than a D50 depending of the maximal response.

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