

Cellular Tests in the Diagnosis of Drug Hypersensitivity

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Abstract: The application of flowcytometry in the study of basophil activation for the diagnosis of allergic diseases has given interesting results in recent years. The quantification of basophil activation by flowcytometry has been proven to be a useful tool for the assessment of the immediate-type response to allergens mediated by IgE or by other mechanisms in drug allergic patients.

Up to now, most basophil activation test studies reported in the literature have used CD69 or CD203c as markers to quantify basophil activation after antigen-specific stimulation. Some technical variations such as the use of whole blood or isolated leukocytes, the addition of IL-3, the conditions of storage of the blood sample, the time of incubation with allergens and their concentration can affect the results of the basophil activation tests.

The basophil activation test is more sensitive and specific than other *in vitro* diagnostic techniques in drug allergy. In various studies, its sensitivity in allergy to muscle relaxant drugs ranges between 36 and 97.7%, with a specificity around 95%. For betalactam antibiotics, basophil activation test sensitivity is 50% and its specificity 90%. For NSAIDs, sensitivity varies between 66% and 75%; specificity is about 93%.

Basophil activation test reproduces *in vitro* hypersensitivity mechanisms involved in immediate-type allergic reactions, allows the diagnosis of allergic and pseudo-allergic reactions particularly for drugs, which are often not detectable by serological techniques, such as determination of specific IgE.

Key Words: Drug allergy diagnosis, cellular tests, basophil activation test, CD63, sulphidoleukotrienes, betalactam allergy, NSAID hypersensitivity.

INTRODUCTION

The *in vitro* diagnostic of allergic diseases has changed completely with the use of some technologies such as flowcytometry, that allows analysis of different cellular lineages even when they are poorly represented in peripheral blood, as it happens with basophils that together with the mast cells, are the primary effector cells of IgE- and non-IgE mediated diseases [1].

The application of flowcytometry in the study of basophil activation (basophil activation test: BAT) for the diagnosis of the allergic diseases has given interesting results in recent years [2-10].

The basis of these assays is in a first step the identification of basophils by specific fluorescent antibodies such as anti-IgE, anti-CD123 (IL-3 receptor) and anti-HLA-DR or anti-CCR3 and in a second step the demonstration of certain membrane phenotypes that appear after exposition to allergen. Up-regulation has been described for expression of the CD45 [11], CD11b and CD11c molecules but down-regulation in the CD62L expression [12], as well as decrease in the mean fluorescent intensity of IgE-carrying basophils after *in vitro* stimulus with the allergen [13].

It has been recently shown that up-regulation of CD63 expression is closely accompanied by and dependent on p38

MAPK phosphorylation [14]. Although some studies show the usefulness of the expression marker CD203c [7], most studies use CD63 [15-17]. There are other potential markers of basophil activation, such as the novel basophil identification antigen, CRTH2 (DP2) and the activation markers CD13, CD164 and CD107a the application of which is being developed [8].

After the binding of a bi- or polyvalent antigen to the high affinity IgE-receptors on the basophil surface, and after an activation process of the cellular membrane, the mediators release and the CD63 expression on its cytoplasmatic membrane are activated.

The basophils release the content of their granules after an activation process dependent on the antigenic stimulus; among the mediators released in this process, the histamine and the antigen-specific sulphidoleukotriene (sLT) production can be quantified simultaneously.

The CD63 molecule is a tetraspan, 53 kDa granular protein that is expressed not only on basophil granules but also on monocytes, macrophages and platelets. The expression of this marker correlates with degranulation and histamine release, which makes it an ideal marker of basophil activation [18]. For *in vitro* stimulation with allergen, the peripheral blood cells are incubated with the suspected allergen for 15-40 minutes at 37°C. After stopping the reaction, the cells are labelled with anti-CD63-PE and anti-IgE-FITC monoclonal antibodies. Two controls are used: a negative control in which the cells are incubated with the stimulation buffer used in the assay and that often contains IL3 (negative con-

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trol alias basal stimulation); as a positive control, an anti-IgE or an anti-IgE receptor antibody can be used.

The interpretation of the results requires the use of some positivity criteria that include both the basophil response to the allergen and its response in basal conditions, without stimulus. The cut-off must be established by the assessment of the optimal values of sensitivity and specificity for each allergen by means of ROC curves [18].

Different studies confirm the diagnostic usefulness of this tool for the *in vitro* allergologic diagnosis [2, 6, 19] and has been validated clinically for various allergens: such as inhalants [17, 20, 21], hymenoptera venoms [16, 22], latex [23-25], muscle relaxants [26, 27], betalactam antibiotics [28-30], pyrazolones [31, 32], and non steroidal anti-inflammatory drugs (NSAIDs) [32, 33].

The application of this technique to the molecular diagnosis, using purified natural or recombinant allergens, that cannot be tested by *in vivo* tests due to safety reasons, gives a new interest dimension to this technique [34, 35].

Its application to the monitoring of antigen-specific immunotherapy [36], in food allergy diagnosis [15, 37, 38] and in chronic urticaria [39, 40-45] is also of interest.

This technique also offers a good correlation with other *in vitro* diagnostic techniques such as histamine release and/or sLT determinations [4, 5, 17, 46-50], but BAT seems more sensitive and specific than the other tests [5, 15]. Anyway we must be cautious in believing that the same rules apply strictly to all outcomes of basophil activation. Even if the initial molecular trigger may be the same, there are enough complex regulation steps along the various different activation cascades, to explain different outcomes, as has been well documented [51-58]. In a number of cases and according to the mode of activation expression of CD63, LTC4 production and histamine release may be entirely dissociated [10]. These differences, sometimes due to a different sensitivity to the external calcium concentration, provoke the paradoxical response observed occasionally among the results obtained by the different techniques [18].

Regarding the possible correlation with the specific IgE determination, the basophil activation can be relatively independent from the IgE extracellular concentration [55].

It is precisely in the field of drug allergy where this test opens an important diagnostic via, either on its own or combined with other techniques [10, 50], allowing avoidance of challenge test in an important number of cases.

Nevertheless, this technique should be considered complementary to skin tests and allergen specific IgE determination, being particularly useful in those situations when these conventional techniques cannot be used or give us doubtful results in comparison with the clinical history of the patient.

BAT IN DRUG ALLERGY

Hypersensitivity Reactions to Neuromuscular Blocking Agents

In the chapter of hypersensitivity reactions to muscle relaxants we can find some studies that prove a high specificity of the technique reaching up to 100% with a sensitivity

between 54% and 91% for CD63 expression [26, 27, 59-62]. This sensitivity is lower (36%) when the marker CD203c is used [60].

Abuaf *et al.* [26] studied basophil activation to muscle relaxants, such as suxamethonium, gallamine, vecuronium, and pancuronium. In a series of 41 patients, they observed a characteristic dose-response curve, but indicate that high drug concentrations can act as unspecific basophil activators. The sensitivity of the technique reported by them is 64% and the specificity 93%.

In another study by Monneret *et al.* [27], a sensitivity of BAT of 54 % was observed with a specificity of 100%. Sudheer *et al.* [60] compared the CD63 and CD203c expressions and measured histamine release in 21 patients who were referred with possible peri-operative anaphylaxis. The sensitivity of CD63, CD203c, basophil histamine release and skin prick test for the muscle relaxants was found to be 79%, 36%, 36% and 64%, respectively.

Kvedarine *et al.* [61] observed in 47 patients a sensitivity of 36% and a specificity of 93% performing BAT (CD63 in whole blood). On the other hand, Ebo *et al.* [62] reported the sensitivity of a similar BAT technique to be 91.7% and the specificity of 100% for rocuronium in a study including 14 patients with anaphylaxis reaction to drug and with positive skin test to rocuronium.

Sensitivity apparently increases up to 80% when BAT is performed shortly after the clinical reaction [61]; this technique allows evaluation of the cross-reactivity between different muscle relaxants [62].

Hypersensitivity to Betalactam Antibiotics

The sensitivity of BAT in the diagnosis of betalactam hypersensitivity immediate reactions is situated in 50% [28, 29, 48], although there are some differences depending on the technique applied (eg. use of total blood or isolated cells) [18].

Our group studied the diagnostic reliability of the BAT and Cellular Antigen Stimulation Test (CAST) techniques in a series of 81 patients with immediate allergy to betalactams (58 patients with positive and 23 with negative skin tests) and in 30 healthy controls [29, 48]. BAT and sLT by CAST ELISA (Bühlmann Laboratories, Allschwil, Switzerland), as well as specific IgE determination (CAP-FEIA, Phadia, Uppsala, Sweden) were performed.

In betalactam allergic patients with positive skin tests the overall sensitivity of BAT was 50.7% and its specificity 93.3%. Amoxicillin (AX) shows the highest sensitivity and specificity values of all the betalactams tested, with significant differences compared with benzyl-penicillin (BP), ampicillin (AM) and minor determinant mixture (MDM). The best predictive values are obtained with BAT, in comparison with sIgE or CAST. The positive predictive value of BAT was 6.4% and the negative predictive value 97.7%, being AX the antibiotic with the highest predictive values.

Specific IgE determination by CAP (Pharmacia) sensitivity was 36.7% and specificity 83.3%. CAST presented a sensitivity of 47.7% and a specificity of 83.3%. The joint use of CAP (Pharmacia) and BAT allows identification of 65.2% of

the betalactam allergic patients with a specificity of 83.3% [29].

In betalactam allergic patients with negative skin tests BAT was positive in 39.1% with a specificity of 93.3%. CAST was positive in 22.7% with a specificity of 83.3%, and CAP showed a sensitivity of 21.7% with a specificity of 83.3%. The association of the three techniques allows diagnosis in 60.9% of the patients of this group, with a specificity of 70% [63].

Torres *et al.* [28] using BAT found similar results. In their study, BAT with different betalactam allergens was performed in 70 patients with immediate allergic reactions. Sensitivity of BAT was 48.6%, sensitivity of sIgE determination 44.3%. Specificity was 91.3%. Positivity to the betalactam allergens was 28.6% to AX, 21.7% to BP, 20% to penicilloyl polylysine (PPL), 12.5% to AM and 2.2% to MDM. In patients with cephalosporin reactions, BAT to the culprit cephalosporin was positive in 77.7%.

These results have been recently confirmed by a multicentric study involving 10 European groups, 181 betalactam allergic patients and 80 controls [10]. In that study it was also shown that the combination with CAST increases the sensitivity to 62%. The combined use of skin tests, sIgE and BAT increases sensitivity to 85% and the addition of CAST to 95%.

Pyrazolone Hypersensitivity

In a study by our group [50], we assessed the diagnostic reliability of BAT in a group of 26 metamizol allergic patients and in 30 controls compared with other *in vitro* techniques (CAST, CAP). These patients were strictly and only reacting to metamizol and not to other NSAIDs; they also had positive skin tests. It is therefore assumed that they belong to the IgE-mediated group of allergies due to analgesics and not to the NSAID hypersensitivity syndrome. Assuming a prevalence in the general population similar to that of the betalactam antibiotics (1%), the positive predictive value of BAT is 100% and the negative predictive value 99.4%. CAP was negative in the 17 cases tested and CAST offered a sensitivity of 52% with a specificity of 90%.

The joint use of BAT and CAST allows detection of 76% of the cases, whereas the association of BAT, CAST and skin tests only increases slightly the reliability of diagnosis (76.9%).

NSAID Hypersensitivity

Some studies performed in patients diagnosed of NSAID hypersensitivity prove that their leukocytes in peripheral blood are able to induce sLT detectable by CAST [64].

Our group [32, 33] studied 60 patients diagnosed with NSAID hypersensitivity and 30 controls. BAT was performed in all and the production of sLTs by CAST (Bühlmann Laboratories, Switzerland) was determined concomitantly with different NSAIDs: aspirin (ASA), paracetamol, metamizol, diclofenac and naproxen at different concentrations.

In this group of patients, BAT with ASA showed a sensitivity of 43.3% and a specificity of 100%, a positive predic-

tive value of 100% and a negative predictive value of 99.4%. The sensitivity and specificity values were 11.7% and 100% for paracetamol, 15% and 100% for metamizol, 43.3% and 93.3% for diclofenac.

The overall sensitivity for the four NSAIDs combined was 63.3% and the specificity 93.3%. If we limit the drugs tested to ASA and diclofenac for practical reasons, the sensitivity of BAT is 58.3% and the specificity 93.3%. In that study, CAST showed a sensitivity of 38.3% and a specificity of 76.7%.

The joint use of BAT and CAST in this group did not increase significantly the diagnostic reliability of BAT.

These results have been recently confirmed by a large multicentric study including 12 European groups, 150 NSAID hypersensitive patients and 163 controls [10]. In that study, sensitivity of BAT when combining ASA, diclofenac and naproxen was found to be 75% and specificity around 90%. However, it was also observed that the mode of isolation of leukocytes may influence the reactions in ASA-tolerant controls: some normal individuals may also show dose-dependent basophil activation to various NSAIDs, which appears related to their pharmacological COX-1 inhibiting effect. The NSAID hypersensitivity syndrome appears therefore related to a shift in the normal pharmacological dose response. An optimal differentiation *in vitro* by BAT is obtained by testing two concentrations each of ASA, diclofenac and naproxen (so called aspirin/diclofenac/naproxen index).

There is some evidence that the mode of cell preparation (eg. whole blood, buffy coat or isolated plasma leukocytes) may play some role in BAT reactivity to NSAID [65, 66].

In some cases of anaphylactic shock to ASA, an IgE-related mechanism has been suggested as the cause of positive BAT [67]. Diclofenac can cause microscopic basophil degranulation but not CD63 expression [68] although in these studies 8 to 10 fold lower concentrations than the ones required for a positive response in BAT were used.

Hypersensitivity to Different Drugs

The basophil activation test has been found to be useful for the diagnostic confirmation in different hypersensitivity reactions to drugs, such as povidone [69], omeprazol [70], hyaluronidase [71], *Viscum album* [72], gelofusine [73, 74], chlorhexidine [75, 76], hydroxyethyl starch [77], patent blue [78, 79], dexchlorpheniramine [80], bovine serum albumin [81], or recombinant hepatitis B vaccine [82]. The main interest of BAT in such cases is to confirm the diagnosis, particularly when skin testing and/or determination of drug-specific IgE are not available.

The usefulness of this technique has also been proven in the cross-reactivity by carbohydrate determinants [83] and in the monitoring of treatment with anti-IgE [84].

CONCLUSIONS

According to the results published up to now, the basophil activation test (BAT) constitutes a diagnostic tool of interest in the study of immediate-type hypersensitivity reactions to drugs. It reproduces *in vitro* hypersensitivity mecha-

nisms involved in an immediate-type allergic reactions, allows the diagnosis of allergic and pseudo-allergic reactions particularly for drugs, which are often not detectable by serological techniques, such as determination of specific IgE.

The positive experience accumulated through the studies performed by different authors [10] about the different allergens allows stating that this technique will have an important development particularly in the field of drug hypersensitivity reactions, where it allows avoidance of challenge test in an important number of cases. Nevertheless more standardization studies are needed and especial effort is under way in Europe, between the different groups using this technique (First International Allergy Flow Cytometry Workshop, Pamplona, October 19-20 2007).

ABBREVIATIONS

AM	=	Ampicillin
ASA	=	Aspirin
AX	=	Amoxicillin
BAT	=	Basophil Activation Test
BP	=	Benzylpenicillin
CAST	=	Cellular Antigen Stimulation Test
MDM	=	Minor determinant mixture
NSAID	=	Non steroidal anti-inflammatory drug
PPL	=	Penicilloyl polylysine
sLT	=	Sulphidoleukotriene

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