

Basophil activation tests in the evaluation of immediate drug hypersensitivity

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Purpose of review

The aim of this study was to confirm the applicability of the basophil activation test (BAT) in the in-vitro diagnosis of drug allergy reactions.

Recent findings

The results obtained in terms of sensitivity and specificity with BAT are encouraging and in significant number of cases can establish the diagnosis.

Summary

BAT sensitivity in beta-lactam allergy was 50%, and specificity ranged from 89 to 97%. There are several studies to validate the BAT in allergy to muscle relaxants showing a sensitivity ranging from 54 to 64% with a specificity of 100 and 93%. The sensitivity of a test for evaluating immediate allergic reactions to drugs may decrease over time. To date, the BAT is the only in-vitro diagnostic method that has been validated for the diagnosis of both IgE-mediated and hypersensitivity reactions to NSAIDs. With respect to other drugs, they are nonetheless interesting as they include the evaluation of allergy to drugs that cannot be studied by other in-vitro techniques. All these data suggest that although a full validation of the test is required, BAT is a potential diagnostic method for evaluating immediate allergic reactions to drugs and NSAID hypersensitivity reactions.

Keywords

basophil activation test, beta-lactams, drug allergy, in-vitro diagnosis, muscle relaxants, NSAIDs

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Introduction

The basophil activation test (BAT) is a useful tool in the in-vitro diagnosis of immediate hypersensitivity reactions to drugs whether these are caused by IgE-dependent mechanisms or other mechanisms leading to the release of histamine and other mediators of immediate hypersensitivity reactions.

Different cellular markers capable of providing information on the activation processes of effector cells in immediate reactions following their in-vitro stimulation with either allergens or drugs exist. Currently, those most frequently used are CD69 and CD203c. The results obtained in terms of sensitivity and specificity with this technique are encouraging and allow a diagnosis to be made in a not negligible number of cases, thus avoiding the use of challenge tests [1[•],2[•],3].

Origin and background of the basophil activation test

The origin of this technique and its application in the diagnosis of allergies can be traced back to the studies

carried out by Nakagawa *et al.* [4], Knol *et al.* [5], Gane *et al.* [6] and Sainte-Laudy *et al.* [7].

Basophils are effector cells in type I (immediate) hypersensitivity reactions. As it is easy to access them in peripheral blood, they provide an interesting substrate for the study of IgE-mediated hypersensitivity reactions. Interestingly, this is also true for other non-IgE-mediated immediate hypersensitivity reactions, such as hypersensitivity to NSAIDs, in which the mechanisms involved have not been completely defined. The main limitation of the low numbers of basophils in peripheral blood can be overcome with the use of flow cytometry-based techniques, which is capable of recovering a sufficient number of cellular events to obtain a representative sample. In recent years, many studies carried out in the field of the in-vitro diagnosis of drug allergies have been published with very interesting results. In these studies [1[•],2[•],3], basophil activation is evaluated following in-vitro-specific antigen stimulation and the subsequent quantification of molecules or activation markers expressed on the membrane of the basophil, when this becomes activated.

Basophil identification

Analysis of the sample with flow cytometry combines data on both morphologic parameters and those resulting from the addition of mAbs (as specific markers of these cells) attached to fluorochromes such as anti-IgE, anti-CD123 (IL-3 receptor) and antihuman leukocyte antigen (HLA)-DR or antichemokine (C-C motif) receptor 3 (CCR3) [8]. Basophils are marked with mAbs directed against cell activation molecules, which following the in-vitro-specific antigen activation of the basophil are expressed on the basophil membrane, as is the case of CD63 [9–11], or which are upregulated, as is the case of CD203c [12].

Basophil activation test reproducibility

Intra-assay reproducibility assessed by duplicate determinations for different allergens is very high, thus allowing the performance of a single test for each allergen concentration [13–15]. The reproducibility of BAT has been found to be quite remarkable in drug allergy with an intra-assay variation coefficient of 0.89 ($P < 0.0001$) for beta-lactams, 0.99 ($P < 0.0001$) for metamizole and 0.92 ($P < 0.0001$) for selected NSAIDs [8].

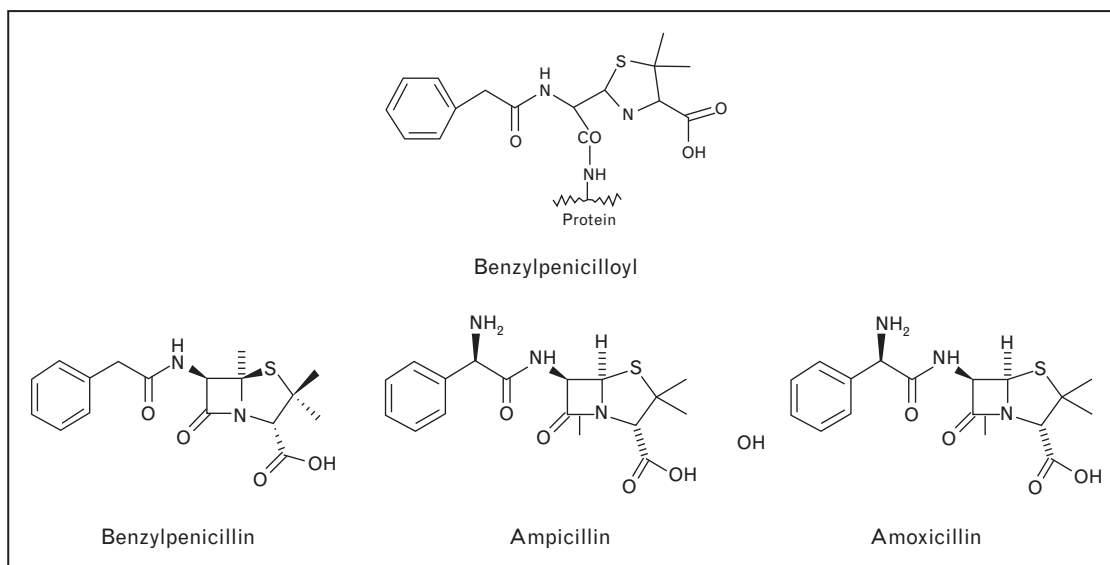
Basophil activation test in immediate-type allergic reactions to beta-lactams

Beta-lactams are the most frequent cause of immediate allergic reactions (IARs) to drugs and are mediated by IgE antibodies. These antibiotics are recognized by the immune system when they bind with carrier molecules producing hapten-carrier conjugates [16]. From these, the best studied is the benzylpenicilloyl (BPO) determi-

nant that is formed after the nucleophilic attack of the beta-lactam ring by the amino group of proteins (Fig. 1) [17]. This determinant conjugated to poly-L-lysine (PLL) has been included in both in-vivo and in-vitro tests for evaluating these reactions. Moreover, there are other penicillin determinants, such as benzylpenicilloic acid and benzylpenicillin, included in the so-called minor determinant mixture (MDM) [16,18]. Although several authors have claimed that penicilloyl polylysine (PPL) and MDM are sufficient to diagnose beta-lactam reactions [19], it has been demonstrated that there is a significant percentage of patients with negative skin tests to those determinants, but positive drug provocation tests (DPTs) [20], and furthermore, there are patients with IAR to amoxicillin with good tolerance to benzylpenicillin. This emphasizes the need to include these determinants, especially when penicillins are the suspected drugs involved in the reaction.

The in-vitro assays are gaining relevance due to the fact that skin tests can induce systemic reactions [21]. Of these, the immunoassays, radioimmunoassay or fluoroenzyme immunoassay have been the most widely used methods. Although they have a lower sensitivity compared with in-vivo tests (skin tests and DPTs), there are patients with positive in-vitro tests but negative skin tests [21]. However, the main requirement for immunoassays is the use of appropriate drug-carrier conjugates (including beta-lactams) that may be difficult to obtain for other drugs involved in IAR. Thus, the advantages of BAT are the ability to work with both conjugated and free drugs and rapidly incorporate new antigenic determinants as soon as they are proved to be involved in the IAR to drugs.

Figure 1 Chemical structures of penicillins and the benzylpenicilloyl group



There are few studies evaluating the role of BAT in the diagnosis of beta-lactam allergic reactions with an appropriate number of patients. In two of these [13,22], similar data were obtained with sensitivity ranging from 48.6 to 50% and specificity from 91.3 to 93.3%. These data demonstrated the reproducibility of the BAT results when populations with similar clinical characteristics and patients with IAR to amoxicillin were analyzed. Moreover, BAT was positive in 14.3% of cases in which skin tests and immunoassay results were negative [22], which can be important in order to avoid the use of drug challenge test.

In a multicenter study performed by the European Network in Drug Allergy (ENDA), results were analyzed from 181 patients with a history of immediate-type allergic reactions to beta-lactams and 81 controls. In this study [23[•]], BAT sensitivity was 50% and specificity ranged from 89 to 97%.

In another study [24], also in patients allergic to amoxicillin, differences were found in the BAT sensitivity depending on the basophil activation markers used: 51% for CD203c and 22% for CD63. Currently, there is no consensus regarding the best activation marker for evaluating these reactions, and further studies need to be carried out in this respect. Of note, the sensitivity obtained in this study for CD63 (22.2%) was lower than previously reported [13,22,23[•]], although in all cases patients were allergic to amoxicillin. These differences in the sensitivity are probably due to the number of antigenic determinants included in each study, as in the work by Abuaf *et al.* [24], only amoxicillin was tested, whereas in the others penicillin G, amoxicillin, PPL, and MDM were also included [13,22,23[•]] (Fig. 1). This suggests that the number of antigenic determinants, including that responsible for the reaction, will influence the sensitivity of the test. In contrast, there are determinants, such as BPO–PLL or ampicillin, which do not increase the sensitivity of the test [13,22].

The combination of the results from BAT and immunoassay increases sensitivity to 65.7% or even to 85% when the results of skin tests were also included without changing the specificity [22,23[•]]. This indicates that the performance of the two in-vitro methods could be a helpful approach to evaluate patients with IAR to beta-lactams, especially in those with a high risk of developing a reaction after the skin tests.

Depending on the clinical response, patients can be classified as selective responders when the patients respond in in-vivo and/or in-vitro tests to amoxicillin with negative skin tests and good tolerance to BPO determinants or cross-reactives when they also react to benzylpenicillin determinants. In many instances, these

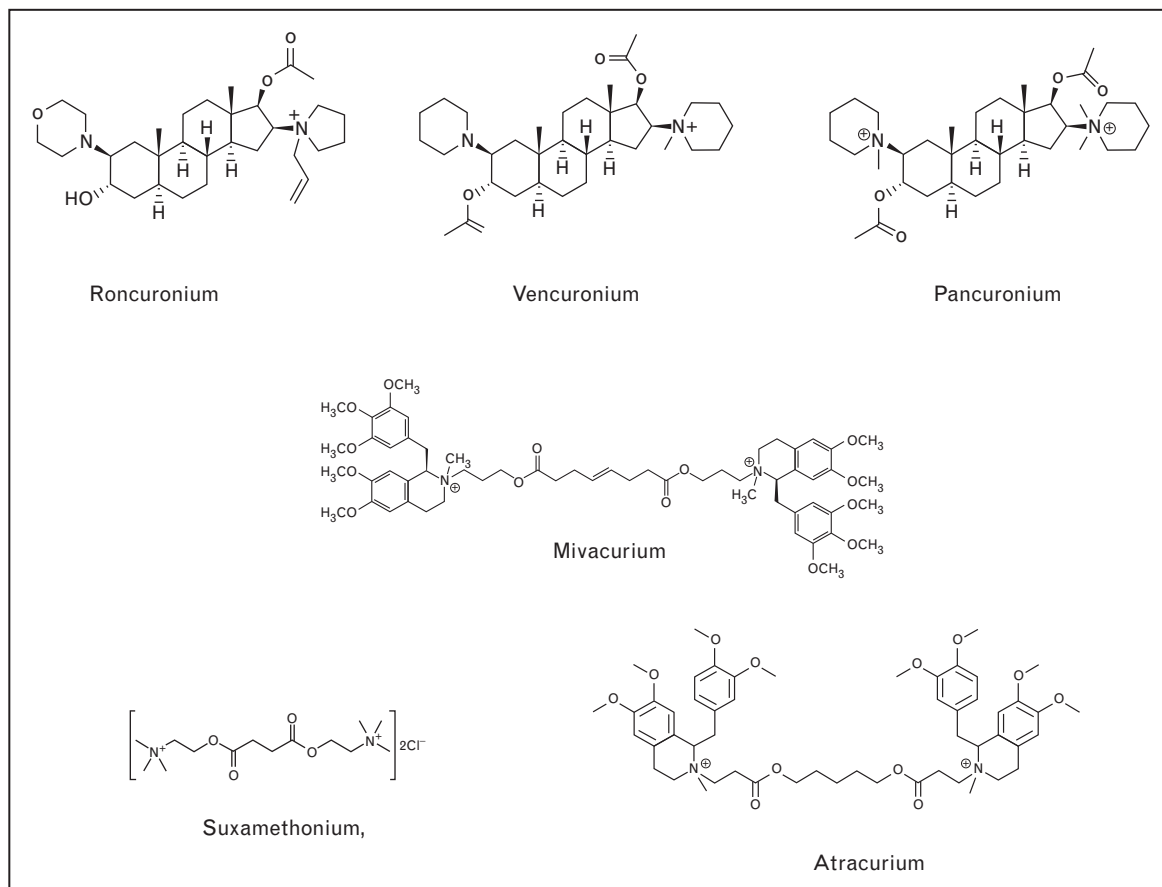
patterns of responses are also supported by the results of immunoassays [25–27]. However, with BAT, it is possible to find positive results when BPO is used as the hapten in patients who are selective responders to amoxicillin [22]. This may limit the use of BAT for defining selective versus cross-responders in patients with immediate hypersensitivity to penicillins. This indicates a certain lack of association between clinical and BAT cross-reactivity that limits the usefulness of applying this method for predicting cross-reactive responses in beta-lactam reactions.

Basophil activation test in allergic reactions to muscle relaxants

Muscle relaxants are an important group of drugs involved in 60% of adverse reactions during anesthesia [28,29]. Although skin test represents an important tool for diagnosing these reactions with a good sensitivity and specificity, they do not answer the clinical questions, probably because muscle relaxants can induce adverse reactions by two different mechanisms: IgE-mediated reactions, mainly to the ammonium group, and nonimmunological reactions [30]. Moreover, as mast cells can react to muscle relaxants differentially depending on the tissue [31], a negative skin test will not confirm a negative reaction of the patient to this drug. These make the evaluation of muscle relaxant reactions a complex issue and highlight the need to use other approaches. There are several early studies [30,32,33] that validated the BAT in muscle relaxant allergy showing a sensitivity ranging from 54 to 64% with a specificity of 100 and 93% when compared with the original clinical reaction. These data are very similar to those obtained for immunoassays that have shown a sensitivity of 62% with a specificity of 100%. Moreover, when the results of both in-vitro methods are combined, the sensitivity can increase to 80% [30]. However, in another study [34], the sensitivity was rather low with a similar specificity (36.1 and 93.3%, respectively) compared with other studies. The explanation of these differences could probably lie in the long time interval elapsed from the acute reaction (mean of 7.6 ± 4 years).

In most of the studies, patients developed IAR to different muscle relaxants, with suxamethonium, rocuronium, mivacurium, vecuronium and pancuronium being those most frequently implicated (Fig. 2). However, when the study was limited to patients with IAR only to rocuronium, the sensitivity of BAT increased to 91.7% with a very high specificity of 100% [35]. This sensitivity was achieved after the choice of an accurate cut-off point by receiver operating characteristic (ROC) analysis was used to determine positive results [35].

The usefulness of BAT in defining cross-reactive responses in patients with muscle relaxant allergy is

Figure 2 Chemical structures of different muscle relaxants

incomplete mainly because these drugs can induce both anaphylactic and anaphylactoid reactions, both of which can be determined by BAT. In the case of IgE-mediated reactions, recognition of the ammonium group depends on different factors related to hydrophobicity and distance with polar groups [36] that may explain the heterogeneity in the pattern of cross-reactivity. It is established that cross-reactivity is more frequent with aminosteroid-derived muscle relaxants, including rocuronium, vecuronium, and pancuronium (Fig. 2), than with benzylisoquinoline-derived muscle relaxants [35]. Different studies have shown that cross-reactivity is frequent and can be detected by both skin tests and BAT. The two techniques are complementary as BAT can identify some cross-reactions not detected with skin tests and vice-versa [35].

Basophil activation test in hypersensitivity to NSAIDs

Hypersensitivity to NSAIDs represents one of the most frequent immediate hypersensitivity drug reactions. The majority of these reactions correspond to a pharmacologic mechanism resulting from the inhibition of cyclooxygenase 1 (COX-1) that leads to a decrease in the synthesis

of prostaglandin E₂ (PGE₂), which in turn hampers the production of sulphidoleukotrienes. Consequently, NSAIDs lead to an increase in the synthesis and release of these and other mediators responsible for the cutaneous and respiratory manifestations that are characteristic of these reactions, for example, cutaneous reactions (urticaria and/or angioedema) and respiratory reactions (rhinitis and/or bronchospasm) [37,38]. Together with these reactions, which are generally the most frequent, there is a lower percentage of reactions to selective NSAIDs of a single type or family of drugs that are IgE-mediated.

Until recently, no in-vitro diagnostic methods existed that had been validated for the diagnosis of this type of allergic reaction to NSAIDs. To date, the BAT is the only in-vitro method that has been applied for the evaluation of both IgE-mediated reactions and the NSAID hypersensitivity reactions, although further studies are needed.

IgE-mediated selective reactions to NSAIDs

These patients react exclusively to only one NSAID family of drugs through an IgE-mediated mechanism,

tolerating other NSAID groups. On the contrary, the nonselective reactors to NSAIDs react with different NSAID groups (i.e. salicylates and pyrazolones and aryl acetics groups) through a non-IgE-mediated mechanism.

Until now, only two studies [14,39^{*}] have been published on selective NSAID reactors. The individuals in the first study were 26 patients with exclusive IgE-mediated allergy to metamizol. In that study, BAT had a sensitivity of 42.3% and an excellent specificity of 100%. Upon adding the leukotriene release test, the sensitivity increases to 52% in the overall patient group with a specificity of 92% [39^{*}]. In another recent study [14] that studied 51 patients with immediate reactions also to metamizol, the sensitivity was 54.9% with a lower specificity of 85.71%. The differences in the sensitivities and specificities of both studies may have been due to differences in the time interval between the in-vitro test being performed and the reaction (16.9 versus 8 months, respectively) and the number of cases with anaphylaxis (57.5 versus 74.5%).

Importantly, the BAT was positive in 38.5% of patients with negative skin tests, but even more importantly, the BAT was positive in 75% of patients with anaphylaxis and negative skin tests. As is the case with other drugs, if the tests are carried out within 6 months from the initial drug reaction, the efficacy of the BAT increases to 56% [14].

NSAID hypersensitivity reactions

The BAT could represent an alternative for the evaluation of these patients and allows the potential dangers of challenge tests to be avoided, although further validation is needed. In the first study published [15], 60 patients with this syndrome were studied using BAT with four NSAIDs: aspirin, metamizol, diclofenac and naproxen. The sensitivity and specificity values are shown in Table 1. With regard to its clinical usefulness, the use of only two drugs (aspirin and diclofenac) allowed a diagnosis to be reached in 58% of these patients with a specificity of 90%. In similar studies [40] carried out in 43 patients with NSAID hypersensitivity, the BAT achieved a sensitivity of 43% with a specificity of 100%.

Subsequently, the European Network for Drug Allergy began a multicenter study [41^{*}] to validate the real usefulness of this technique. It must be stated that a heterogeneous response in the results from each participating group in both sensitivity and specificity was observed. A detailed analysis of these differences is beyond the scope of this review but can be attributed to two main reasons: different clinical characteristics in patients studied and technical variations in the way the test was carried out among the different groups. One hundred and forty patients were evaluated with the same

Table 1 Sensitivity and specificity of basophil activation test in NSAID hypersensitivity

	BAT sensitivity		BAT specificity	
	Ref. 15 [*] (%)	Ref. 40 (%)	Ref. 15 (%)	Ref. 40 (%)
Aspirin	43	39	100	87
Paracetamol	11	9	100	94
Metamizol	15	10	100	90
Diclofenac	43	43	93	79
Naproxen	55	42	74	88

ASA, acetylsalicylic acid; BAT, basophil activation test.

^{*} All the patients selected in the study by Gamboa *et al.* [15] experienced acute episodes of urticaria–angioedema or dyspnea within the first 4 h after NSAID administration. When the symptoms occurred at two or more occasions after administration of at least two NSAIDs belonging to different pharmacological families, the diagnosis of hypersensitivity syndrome to NSAIDs was considered as established. Provocation was not done in these patients because the diagnosis was considered as clinically firmly established. Four patients were nevertheless challenged with ASA in spite of having suffered several adverse reactions with different NSAIDs.

four NSAIDs as in the previous study as well as naproxen (data not published). The patients were diagnosed with NSAID hypersensitivity based on a history of at least two clinical events with one or more NSAIDs in all and positive provocation test to acetylsalicylic acid (ASA) 500 mg, in addition, in 107 of 140 (76%) of these patients. Paracetamol and metamizol added nothing to the diagnostic yield. Thus, with the use of aspirin, diclofenac and naproxen, a sensitivity of 64% was achieved both in patients with hypersensitivity confirmed by oral challenge (63%) and in patients presenting with reactions to multiple NSAIDs (65%) with a specificity of 60%. This specificity value was notably better in those groups who used buffy coat than in those using plasma leukocytes. This fact, together with the positivity index calculated on baseline activation, following stimulation with the three NSAIDs mentioned above increased specificity to 96–98% [1^{*}]. However, lower values in sensitivity and specificity have been found by others (data not shown). These increase the need for performing further collaborative studies using identical laboratory methods.

In conclusion, although technical standardizations of the BAT still remain to be performed, this test is the only technique currently available that allows the evaluation of patients with NSAID hypersensitivity syndrome or selective IgE-mediated reactions to metamizol without the use of challenge tests and their associated dangers for patients with sufficiently high sensitivity and specificity.

Basophil activation test in allergic reactions to other drugs

With respect to other drugs, there are few studies and most have had a limited number of cases. Despite this, they are nonetheless interesting as they include the

evaluation of allergy to drugs that cannot be studied by other in-vitro tests such as immunoassays. Cases involving IAR to corticosteroids [42], heparins [43,44] and omeprazole [45] have demonstrated the usefulness of BAT in evaluating IAR to these drugs. BAT has also been used to evaluate allergy to cyclosporine [46], gelofusine [47], dexchlorpheniramine [48], chlorhexidine [49], iodinated povidone [50] hyaluronidase [51] or BSA [52].

Relationship between basophil activation test sensitivity and time interval from the acute reaction

The sensitivity of a test for evaluating IAR to drugs can decrease over time as has been demonstrated in skin tests sensitivity in beta-lactam allergic patients [53]. This underlines the importance of carrying out these tests close after the acute reaction.

The loss of sensitivity in BAT has been observed first in patients with muscle relaxant allergic reactions [32], in beta-lactam allergic patients [54^{*}] and recently in dipyrone allergic reactions [39^{*}]. In the first study [32], the sensitivity of BAT to muscle relaxants was shown to be very high (85.7%) in patients who had the reaction less than 3 years previously. However, it decreased dramatically to 47.6% when the study was performed 4–8 years after the reaction had occurred. In the other study [54^{*}], the rates of tests becoming negative for specific IgE to penicillins were analyzed in both BAT and the radioallergosorbent test (RAST). Data showed that 1 year after the reaction, only 12.2% of patients for BAT and 22% for RAST remained positive, and the rate of in-vitro tests turning negative was faster for BAT. The authors also showed that contact with the antigen, as occurs during the skin tests, can maintain the in-vitro response in RAST but not in BAT [54^{*}]. The follow-up of patients with positive BAT to metamizol showed that 60% of these patients became negative after 6 months [39^{*}].

On the basis of these data, it is advisable to evaluate patients with IAR to drugs near the time the reaction occurs. However, at least 2 weeks should elapse after the acute phase in order to avoid the presence of a high percentage of activated basophils in basal conditions [55]. Moreover, in-vitro tests becoming negative is not always accompanied by good tolerance to the drugs, although this is difficult to confirm because a new contact with the drug could have an influence on resensitization [56].

All these data suggest that although a full validation of the test is required, BAT is a useful diagnostic method for evaluating IAR to drugs, and the combination with other in-vivo and in-vitro methods will improve the sensitivity and the knowledge of well tolerated alternatives.

Conclusion

The BAT constitutes a valuable diagnostic tool of interest in the study of immediate hypersensitivity reactions to drugs. It reproduces in-vitro hypersensitivity mechanisms involved in immediate-type allergic reactions. It also 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. In addition, several different drugs can be tested simultaneously with a small amount of blood, reducing the need for DPTs. International collaboration would be useful in facilitating a consensus on the use of BAT in evaluating immediate drug allergies.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

- 1 Sanz ML, Gamboa PM, De Weck AL. Cellular tests in the diagnosis of drug hypersensitivity. *Curr Pharm Des* 2008; 14:2803–2808.
BAT reproduces in-vitro hypersensitivity mechanisms involved in an immediate-type allergic reaction and allows the diagnosis of allergic and pseudo-allergic reactions, particularly for drugs that are often not detectable by serological techniques such as determination of specific IgE.
- 2 de Weck AL, Sanz ML, Gamboa PM, *et al.* Diagnostic tests based on human basophils: more potentials and perspectives than pitfalls. *Int Arch Allergy Immunol* 2008; 146:177–189.
Our experience of several years with clinical validation of various applications of BAT shows that these new technologies have more potential than pitfalls.
- 3 Sanz ML, Gamboa PM, García-Avilés C, De Weck A. Drug hypersensitivities: which room for biological tests? *Eur Ann Allergy Clin Immunol* 2005; 37:230–235.
- 4 Nakagawa T, Stadler BM, De Weck AL. Flow-cytometric analysis of human basophil degranulation. I. Quantification of human basophils and their degranulation by flow-cytometry. *Allergy* 1981; 36:39–47.
- 5 Knol EF, Mul FJP, Jansen H, *et al.* Monitoring human basophil activation via CD63 monoclonal antibody 435. *J Allergy Clin Immunol* 1991; 88:328–338.
- 6 Gane P, Pecquet C, Lambin P, *et al.* Flow cytometry evaluation of human basophils. *Cytometry* 1993; 14:344–348.
- 7 Sainte-Laudy J, Sabbah A, Vallon C, Guerin JC. Analysis of anti-IgE and allergen induced human basophil activation by flow cytometry: comparison with histamine release. *Inflamm Res* 1998; 47:401–408.
- 8 Sanz ML, Maselli JP, Gamboa PM, *et al.* Flow cytometric basophil activation test: a review. *J Invest Allergol Clin Immunol* 2002; 12:143–154.
- 9 Moneret-Vautrin DA, Sainte-Laudy J, Kanny J, Fremont S. Human basophil activation measured by CD 63 expression and LTC4 release in IgE-mediated food allergy. *Ann Allergy Asthma Immunol* 1999; 82:33–40.
- 10 Sainte-Laudy J, Drouet M, Lauret MG, *et al.* Basophil activation test by flow cytometry in hymenoptera venom allergy. *Allergie Immunologie* 1999; 31:11–14.
- 11 Sanz ML, Sánchez G, Vila L, *et al.* Ag-induced basophil activation: CD63 cell expression detected by flow cytometry in patients allergic to Dermatophagoides pteronyssinus and Lolium perenne. *Clin Exp Allergy* 2001; 31:1–8.
- 12 Boumiza R, Debard AL, Monneret G. The basophil activation test by flow cytometry: recent developments in clinical studies, standardization and emerging perspectives. *Clin Mol Allergy* 2005; 3:3–9.

- 13 Sanz ML, Gamboa PM, Antépara I, *et al.* Flow-cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to beta-lactam antibiotics. *Clin Exp Allergy* 2002; 32:277–286.
- 14 Gamboa PM, Sanz ML, Caballero MR, *et al.* Use of CD63 expression as a marker of in vitro basophil activation and leukotriene determination in metamizol allergic patients. *Allergy* 2003; 58:312–317.
- 15 Gamboa P, Sanz ML, Caballero MR, *et al.* The flow-cytometric determination of basophil activation induced by aspirin and other nonsteroidal and anti-inflammatory drugs (NSAIDs) is useful for in vitro diagnosis of the NSAID hypersensitivity syndrome. *Clin Exp Allergy* 2004; 34:1448–1457.
- 16 Blanca M, Torres MJ, Mayorga C, *et al.* Immediate allergic reactions to betalactams: facts and controversies. *Curr Opin Allergy Clin Immunol* 2004; 4:261–266.
- 17 Batchelor FR, Dewdney JM, Gazzard D. Penicillin allergy: the formation of the penicilloyl determinant. *Nature* 1965; 206:362–364.
- 18 Sullivan TJ, Wedener HJ, Shatz GS, *et al.* Skin testing to detect penicillin allergy. *J Allergy Clin Immunol* 1981; 68:171–180.
- 19 Macy E, Richter PK, Fakoff R, Zeiger R. Skin testing with penicilloate prepared by an improved method: amoxicillin oral challenge in patients with negative skin test responses to penicillin reagents. *J Allergy Clin Immunol* 1997; 100:586–591.
- 20 Torres MJ, Mayorga C, Leyva L, *et al.* Controlled administration of penicillin to patients with a positive history but negative skin and specific serum IgE tests. *Clin Exp Allergy* 2002; 32:270–276.
- 21 Torres MJ, Romano A, Mayorga C, *et al.* Diagnostic evaluation of a large group of patients with immediate allergy to penicillins: the role of skin testing. *Allergy* 2001; 56:850–856.
- 22 Torres MJ, Padial A, Mayorga C, *et al.* The diagnostic interpretation of basophil activation test in immediate allergic reactions to beta-lactams. *Clin Exp Allergy* 2004; 34:1768–1775.
- 23 De Weck AL, Sanz ML, Gamboa PM, *et al.* Diagnosis of immediate-type Beta-lactam allergy in vitro by flowcytometric basophil activation test (BAT alias FLOW-CAST) and sulfidoleukotriene production (CAST-ELISA): a multicentric study. *J Invest Allergol Clin Immunol* 2009; 19:91–109.
- In a multicenter study encompassing 10 European centers, 181 patients with history of immediate beta-lactam allergy and 81 controls, we have evaluated the diagnostic efficiency of specific IgE determinations and of two cellular tests based on basophil reactivity test, the BAT.
- 24 Abuaf N, Rostane H, Rajoely B, *et al.* Comparison of two basophil activation markers CD63 and CD203c in the diagnosis of amoxicillin allergy. *Clin Exp Allergy* 2008; 38:921–928.
- 25 Blanca M, Vega JM, Garcia J, *et al.* Allergy to amoxicillin with good tolerance to other penicillins: study of the incidence in patients allergic to beta-lactams. *Clin Exp Allergy* 1990; 20:475–481.
- 26 Blanca M, Mayorga C, Torres MJ, *et al.* Clinical evaluation of Pharmacia CAP System RAST FEIA amoxicilloyl and benzylpenicilloyl in patients with penicillin allergy. *Allergy* 2001; 56:862–870.
- 27 Vega JM, Blanca M, Carmona MJ, *et al.* Delayed allergic reactions to beta-lactams: four cases with intolerance to amoxicillin and/or ampicillin and good tolerance to penicillin G and V. *Allergy* 1991; 46:154–157.
- 28 Moss J. Muscle relaxants and histamine release. *Acta Anaesthesiol Scand* 1995; 39 (S106):7–12.
- 29 Laxenaire MC. Epidemiology of anesthetic anaphylactoid reactions: fourth multicenter survey. *Ann Fr Anesth Reanim* 1999; 18:769–809.
- 30 Monneret G, Benoit Y, Debard AL, *et al.* Monitoring of basophil activation using CD63 and CCR3 in allergy to muscle relaxant drugs. *Clin Immunol* 2002; 102:192–199.
- 31 Stellato C, de Paulis A, Crillo R, *et al.* Heterogeneity of human mast cells and basophils in response to muscle relaxants. *Anesthesiology* 1991; 74:1078–1086.
- 32 Abuaf N, Rajoely B, Ghazouani E, *et al.* Validation of flow cytometry assay detecting in vitro basophil activation for the diagnosis of muscle relaxant allergy. *J Allergy Clin Immunol* 1999; 104:411–418.
- 33 Sudheer PS, Hall JE, Read GF, *et al.* Flow cytometric investigation of peri-anesthetic anaphylaxis using CD63 and CD203c. *Anaesthesia* 2005; 60:251–256.
- 34 Kvedariene V, Kamey S, Ryckwaert Y, *et al.* Diagnosis of neuromuscular blocking agent hypersensitivity reactions using cytofluorimetric analysis of basophils. *Allergy* 2006; 61:311–315.
- 35 Ebo DG, Bridts CH, Hagendorens MM, *et al.* Flow-assisted diagnostic management of anaphylaxis from rocuronium bromide. *Allergy* 2006; 61:935–939.
- 36 Mertes PM, Aimone-Gastin I, Guéant-Rodriguez RM, *et al.* Hypersensitivity reactions to neuromuscular blocking agents. *Curr Pharm Des* 2008; 14: 2809–2825.
- 37 Szczeklik A, Stevenson D. Aspirin-induced asthma: advances in pathogenesis, diagnosis and management. *J Allergy Clin Immunol* 2003; 111:913–921.
- 38 Stevenson D. Aspirin and NSAID sensitivity. *Immunol Allergy Clin North Am* 2004; 24:491–505.
- 39 Gómez E, Blanca-Lopez N, Torres MJ, *et al.* IgE-mediated immediate allergic reactions to dipyrone: value of basophil activation test in the identification of patients. *Clin Exp Allergy* 2009. [Epub ahead of print]
- In this study, the value of BAT in the evaluation of IgE-mediated allergic reactions to pyrazolones is discussed. BAT may be a useful alternative to skin testing, especially in patients with severe reactions who may develop symptoms after skin testing. BAT testing negative is associated with the time of performance of the test after the reaction occurred.
- 40 Rodríguez-Trabado A, Cámara-Hijón C, Ramos-Cantariño A, *et al.* Basophil activation test for the in vitro diagnosis of nonsteroidal anti-inflammatory drug hypersensitivity. *Allergy Asthma Proc* 2008; 29:241–249.
- 41 de Weck AL, Sanz ML, Gamboa PM, *et al.* A multicenter study on the NSAID hypersensitivity syndrome. I. Clinical findings and in vitro diagnostic. *J Invest Allergol Clin Immunol* (in press).
- In this study, we have further analyzed basophil activation *in vitro* in apparently normal individuals tolerating the administration of NSAIDs, in particular the specificity of these reactions and the role of technical conditions such as the cell isolation technique.
- 42 Lehmann S, Ott H. Glucocorticoid hypersensitivity as a rare but potentially fatal side effect of paediatric asthma treatment: a case report. *J Med Case Reports* 2008; 2:186.
- 43 Ebo DG, Haine SE, Hagendorens MM, *et al.* Hypersensitivity to nadroparin calcium: case report and review of the literature. *Clin Drug Investig* 2004; 24:421–426.
- 44 Caballero MR, Fernandez-Benitez M. Allergy to heparin: a new in vitro diagnostic technique. *Allergol Immunopathol* 2003; 31:324–328.
- 45 Gamboa PM, Sanz ML, Urrutia I, *et al.* CD63 expression by flow cytometry in the in vitro diagnosis of allergy to omeprazole. *Allergy* 2003; 58:538–539.
- 46 Ebo DG, Piel GC, Conraads V, Stevens WJ. IgE-mediated anaphylaxis after first intravenous infusion of cyclosporine. *Ann Allergy Asthma Immunol* 2001; 87:243–245.
- 47 Apostolou E, Deckert K, Puy R, *et al.* Anaphylaxis to gelofusine confirmed by in vitro basophil activation test: a case series. *Anaesthesia* 2006; 61:264–268.
- 48 Caceres Calle O, Fernandez-Benitez M. Allergy to dexchlorpheniramine. Study of a case. *Allergol Immunopathol* 2004; 32:306–309.
- 49 Ebo DG, Bridts CH, Stevens WJ. IgE-mediated anaphylaxis from chlorhexidine: diagnostic possibilities. *Contact Dermatitis* 2006; 55:301–302.
- 50 Le Pabic F, Sainte-Laudy J, Blanchard N, Moneret-Vautrin DA. First case of anaphylaxis to iodinated povidone. *Allergy* 2003; 58:826–827.
- 51 Ebo DG, Goossens S, Opsomer F, *et al.* Flow-assisted diagnosis of anaphylaxis to hyaluronidase. *Allergy* 2005; 60:1333–1334.
- 52 Orta M, Ordoqui E, Aranzabal A, *et al.* Anaphylactic reaction after artificial insemination. *Ann Allergy Asthma Immunol* 2003; 90:446–451.
- 53 Blanca M, Torres MJ, Garcia JJ, *et al.* Natural evolution of skin test sensitivity in patients allergic to beta-lactams. *J Allergy Clin Immunol* 1999; 103:918–924.
- 54 Fernandez TD, Torres MJ, Blanca-López N, *et al.* Negativization rates of IgE radioimmunoassay and basophil activation test in immediate reactions to penicillins. *Allergy* 2009; 64:242–248.
- This is the first work studying the rate of negativization of two in-vitro tests for determining IgE antibodies to penicillins. Conversion to negative took longer for the RAST assay, although the differences were only detected with the amoxicillin hapten. Skin testing influenced the rate of negativization of the RAST assay, contributing to maintenance of in-vitro sensitivity. These data highlight the importance of evaluating the allergic patients closer to the time the reaction occurred.
- 55 Rodríguez Trabado A, Cámara Hijón C, Porcel Carreno SL, *et al.* Anaphylaxis caused by cloxacillin: diagnosis with seriated analysis by way of basophil activation test. *Allergy Asthma Proc* 2006; 27:269–272.
- 56 Antúnez C, Fernández T, Blanca-Lopez N, *et al.* IgE antibodies to betalactams: relationship between the triggering hapten and the specificity of the immune response. *Allergy* 2006; 61:940–946.