

EDITORIAL

Basophil histamine release tests in the diagnosis of allergy and asthma

Identification of the precipitating factors in allergy and asthma enables allergen avoidance strategies and the prevention of life-threatening anaphylactic reactions. In clinical practice, measurement of allergen specific IgE in serum and skin prick testing (SPT) are the most commonly used tests, although neither offers 100% specificity and sensitivity. In some patients there are discrepancies between serological results and clinical symptoms [1]. Many agents may cause pseudo-allergic reactions, where although the clinical symptoms are similar to true allergic reactions an immune mediated process is not involved or its involvement has not been demonstrated (cf. intrinsic asthma and some drug-induced adverse reactions). Furthermore, skin prick testing is not suitable for all patients and may cause clinical symptoms (up to anaphylactic shock in some patients) as can other tests, e.g. bronchoprovocation or oral challenge with the suspected allergen.

In an attempt to find more sensitive and specific tests to investigate allergic and asthmatic reactions the analysis of *in vitro* mediator release from effector cells has been used. Although both mast cells and basophils are known to be important in these reactions, the tissue location of mast cells has made such studies difficult. Thus for mast cell studies, nasal or bronchoalveolar lavage cells or biopsy material may be employed but these methods require considerable expertise and cannot be used routinely. Consequently, basophils remain the cells of choice. Basophil histamine release studies have been described in the literature since the 1960s [2], where allergen-induced basophil histamine release was described as a more accurate index of clinical severity than antibody measurements. However, such investigations have not become part of routine practice nor have there been many commercially available applications. Why is this the case? Part of the problem lies in the variations in the methods used in the literature: some protocols use whole blood, others leucocyte preparations and yet others require purified basophil populations. When it comes to histamine assays the choice is even greater: radioenzymatic assays, HPLC, radioimmunoassays, ELISAs, fluorescent assays (with or without purification of the sample, manual or automated, glass fibre based assays). When investigating drug-induced basophil activation, the drug itself may exert unwanted effects on the assay, e.g. neuromuscular blocking agents interfere with the radioenzymatic assay. More recently, methods have been described which measure the release of leukotrienes from blood/basophil preparations or identify increased expression of surface markers on

basophils after allergen or drug challenge using flow cytometry. In the following article, we compare published results using the various methods and the few commercially available test systems. In all cases, we will concentrate on the factors that are important for the routine analysis of allergic and asthmatic reactions including:

- high sensitivity and specificity; and
- ease of use in the clinical setting.

Non-commercial assays for determination of basophil activation

Histamine and tryptase release assays

It has long been known that some patients have non-responding basophils, i.e. there is no mediator release following allergen challenge [3]. Nolte et al. have advocated the use of passive sensitization of donor basophils with plasma samples from the subjects under investigation, followed by stimulation of the donor basophils with the relevant allergen [4]. Using plasma samples from extrinsic asthmatic children, who had undergone bronchoprovocation tests, skin prick tests (SPT) and RAST, they found complete agreement in 10/18 patients (56%). In 94% of the subjects the results from the passive sensitization histamine release method agreed with the RAST, in 83% with the bronchoprovocation and in 78% with the skin tests; these values were slightly lower when direct histamine release was used, namely 74%, 63% and 72%. Passive sensitization has an important advantage in routine clinical use, namely plasma preparation can be done anywhere and the more technically challenging basophil liberation test can be performed in a specialized laboratory. It can also overcome the problem of age-dependent reduction in histamine release [5].

Table 1 demonstrates the reported sensitivity and specificity of mediator release from basophils in allergy diagnosis. Griese et al. [6] examined 93 extrinsic asthmatic children compared with bronchoprovocation as the gold standard. They found that the histamine liberation test had higher sensitivity and specificity than skin testing or RAST analysis, e.g. for house dust mite skin testing had a sensitivity of 66% and a specificity of 64%, whereas for RAST the values were 76% and 63%, respectively. They reported that test results for histamine release could be obtained within 4–5 h and cost c. \$30 per sample. This paper pointed out one of the major difficulties in histamine

Table 1 Sensitivity and specificity of noncommercial and commercial basophil release tests in allergy diagnosis

Gold standard	Reference	Stimulus	Sensitivity %	Specificity %	<i>n</i>
Bronchoprovocation					
	6	HDM	87	84	69
		Mould	90	86	24
		HDM or mould	87	85	93
History + skin test + provocation (some cases)	7	Drugs	51	63	108
Case history, skin test and Specific IgE	8*	HDM	75	63	384
		Anti-IgE	88	30	384
		Spontaneous	84	34	384
DBPCFC	9	Commercial food allergens	56	78	17
		Fresh food allergens	89	78	17
Clinical history + skin test + provocation (unless anaphylactic shock)	12	Food allergens	85	100	43
Skin test	15	Grass extracts	87	85	8
Specific IgE		Grass extracts	83	87	8
Oral challenge	16	Food allergens	53	82	n.r
Nasal provocation	17	Allergens (HDM or cedar pollen)	82	67	53
Case history, skin test and specific IgE	26*	Allergen	72	57	468
		Anti-IgE	72	57	468
		Spontaneous	56	55	468
Clinical history	27	Drugs	24	n.r.	18
Bronchoprovocation	28	Anti-IgE	90	78	81
Clinical history	29	Latex	45	n.r.	33

HDM = house dust mite; DBPCFC = double blind placebo controlled food challenge. *Measured tryptase release; n.r. = not reported.

release studies, namely, the appropriate cut-off limit for a positive reaction. The authors used > 20% for house dust mite but only > 5% for moulds. Demoly et al. [7] also reported that the sensitivity and specificity varied markedly with the cut-off for histamine release in drug allergy patients. Using a cut-off of 5% as positive the overall sensitivity was 51.4%, with 10% as the cut-off this dropped to 22.8%; the specificities were 63% and 79.5%, respectively. Receiver operating characteristic curves should be employed in order to determine the appropriate cut-off points [8].

The allergen used in the assays can also cause problems especially when investigating food allergies. Norgaard et al. [9] examined 17 adults with suspected allergy to egg and/or milk, using the double blind placebo controlled food challenge as the gold standard. They compared commercial extracts, fresh standardized foods and extracts from local fresh food. Using commercial extract the sensitivity of the histamine release test was only 56%, with a specificity of 78%; however, the sensitivity increased to 89% without a concomitant loss of specificity when they employed

extracts of fresh local foods. This increase in sensitivity also occurred in the skin test (commercial: sensitivity 75%, specificity 77%; fresh food: sensitivity 100%, specificity 77%). In laboratory animal allergies Hook et al. [10] found that there was good agreement between histamine release and skin prick test results, providing the skin test response was strongly positive.

Sulphidoleukotriene release assays

Allergy diagnoses using sulphidoleukotriene release (Table 2) have mainly employed the commercial assays and the allergens provided (see below). However, the paper by Abrahamsen et al. in this issue uses a novel combined method to differentiate between aspirin-sensitive asthmatics, non-allergic asthmatics without aspirin intolerance, allergic asthmatics and control subjects [11]. After dextran sedimentation, leucocytes were primed with IL-3 prior to challenge with C3a or anti-IgE. Leukotrienes were measured using the Cellular Allergen Stimulation Test, CAST assay. The quotient C5a/anti-IgE induced leukotriene production was calculated.

Table 2 Sensitivity and specificity of noncommercial and commercial leukotriene production tests in allergy diagnosis

Gold Standard	Reference	Stimulus	Sensitivity %	Specificity %	n
Clinical history + skin test + provocation (unless anaphylactic shock)	3	Food allergens	85	100	43
Case history, skin test and Specific IgE	8	HDM	71	87	384
Case history, skin test	21	Honey bee venom	100	77	23
		Yellow jacket venom	83	77	100
Clinical history	22	NSAIDs	63–80	70–100	25
Clinical history and oral challenge	24	Aspirin	18	99	38
		Aspirin + C5a	50	99	
		Aspirin + C5a + NSAIDs	73	97	
Clinical history	29	Latex	43	n.r.	33

HDM = house dust mite; n.r. = not reported.

Using the optimal cut-off point of 1, the quotient allows a diagnostic differentiation of aspirin-sensitive and non-atopic asthmatics from atopic asthmatics and controls with a sensitivity of 73% and a specificity of 94%. In contrast, measuring histamine release did not enable the differentiation of the two groups.

Increased expression of surface markers

The use of flow cytometric analysis of basophil surface markers to investigate allergy is a more recent method. Whole blood, dextran sedimentation or Ficoll separated blood have all been used with comparable results. Although both CD63 (gp53) and CD45 have been used, CD63 seems to be the most specific (93–100% specificity) [12–14]. It is faster (results available within 3 h) and less expensive than measuring leukotriene release from basophils [12]. No statistical difference was observed between spontaneous

and anti-IgE induced basophil activation for samples assayed immediately or 24 h after sampling [12]. However, differences were observed with samples left for 48 h before assay. On first sight, the latter method offers a number of advantages for the routine analysis of allergic and asthmatic reactions. Most clinical immunology services will have access to bench top flow cytometers, which lend themselves to automated analyses. They use methods already employed in the laboratory and hence do not require the acquisition of new equipment or techniques. A summary of reported applications and diagnostic sensitivity and specificity is given in Table 3.

Commercial assays for determination of basophil activation

Historically, basophil functional assays have not been widely used in routine diagnostic laboratory practice.

Table 3 Sensitivity and specificity of noncommercial and commercial measurements of increased expression of surface markers on basophils in allergy diagnosis

Gold standard	Reference	Stimulus	Sensitivity %	Specificity %	n
Clinical history + skin test + provocation (unless anaphylactic shock)	12	Food allergens	80	100	43
Clinical history	13	Drugs	64	93	24
Skin test	14	Allergens	83	100	12
Skin test or nasal challenge	25	Cypress pollen	91	100	65
Clinical history	27	Drugs	71	n.r.	18

n.r. = not reported.

Reasons for this include: (1) the time consuming and labour intensive nature of the assays; (2) the lack of standardization of methodology; (3) the relative paucity of data on assay performance characteristics; (4) issues regarding quality assurance; and (5) cost factors. With the availability of commercial assays, which address a number of these issues, the opportunity to evaluate these assays in the diagnostic laboratory setting now exists.

A limited number of commercial products are currently available (information ascertained from searches of the World Wide Web). Essentially these assays fall into three categories which may be defined on the basis of the method by which basophil activation is assessed, namely: (1) histamine release; (2) sulphidoleukotriene release; and (3) CD63 expression.

Histamine release assays

Assays for the measurement of released histamine from stimulated heparinized blood samples are available from Hycor Biomedical Inc. (<http://www.hycorbiomedical.com>), HY.TEC™ BHR Assay; IBL Immunobiological Laboratories (<http://www.ibl-hamburg.com>) and Pharmacia & Upjohn (<http://www.diagnostics.nu>).

The HY.TEC™ BHR assay (competitive ELISA) may be performed as a manual test or using the company's automated analyser. Activators from a range of environmental allergens, foods, venoms and drugs are provided in a standardized format. The kit is marketed for *in vitro* diagnostic use. A published clinical study indicated good sensitivity and specificity (in the range 90–100%) for grass extracts relative to SPT and specific IgE tests, although the number of subjects tested (8) was very small [15]. Evaluation of the assay in the diagnosis of food hypersensitivity in paediatric patients has been reported [16]. Using oral challenge as the reference method, superior diagnostic specificity (82%) was observed compared to specific IgE (24%), specific IgG (47%) and SPT (32%). Assay sensitivity, however, was less impressive — BHR: 53%; specific IgE (82%), specific IgG (57%) and SPT (82%).

The histamine release assays from IBL Immunobiological Laboratories (ELISA, measuring histamine) and Pharmacia & Upjohn (RIA, measuring methylhistamine) do not provide allergen extracts as part of the kit, thus necessitating the optimization and standardization of reagents in individual laboratories. The performance of these assays in diagnostic trials is awaited.

A commercial glass fibre-based whole blood histamine release test, in which bound histamine is determined by fluorimetry, has been marketed by a number of companies. The histamine release test (HRT) (Asahi Chemical Industry Company, Japan) reportedly shows good correlation and concordance with CAP-RAST results, SPT and nasal

provocation tests [17]. At the time of writing we were unable to ascertain the current availability of this test. The HR-Test supplied by Reflab, Denmark (<http://www.reflab.dk>) offers a range of products which allow determination of histamine either directly in body fluid samples, or following stimulation with standard or customized panels of allergens.

Sulphidoleukotriene release assay

The CAST® ELISA test, recently superseded by the CAST® 2000 ELISA (Bühlmann Laboratories; <http://www.bulhmannlabs.ch>) allows the quantitative determination of sulphidoleukotrienes following leucocyte stimulation. This is a three-stage assay involving: (1) leucocyte isolation by dextran sedimentation; (2) simultaneous leucocyte priming and stimulation with IL-3 and allergen; and (3) measurement by ELISA of synthesized sulphidoleukotriene LTC₄ and metabolites LTD₄ and LTE₄. A comprehensive range of individual allergens (inhalants; foods; drugs) is available from the company in addition to several allergen mixes (e.g. TOP-CAST® and TOP-CAST® Paediatric Mix). The test is marketed for *in vitro* research.

The CAST® ELISA has been investigated in a number of clinical situations. Medralla *et al.* [18,19] have reported positive sulphidoleukotriene release in patients with grass pollen and mite (*Dermatophagoides pteronyssinus*) allergy, although rigorous criteria for evaluation of the assay were not applied. In a study reported by Ferrer *et al.* [20], *D pteronyssinus*-induced sulphidoleukotriene production in mite sensitive patients was shown to be positively correlated with histamine release ($r = 0.83$; $P < 0.001$), with weaker correlations observed for SPT ($r = 0.49$) and specific IgE levels ($r = 0.4$). Similar findings were made by Rossi *et al.* [8] in a series of 247 mite sensitive patients. The assay appeared to offer good diagnostic performance for *D pteronyssinus* and *D farinae* with positive predictive values quoted of 88% and 91%, respectively.

In patients with insect venom allergy, the CAST® ELISA assay has been reported as offering better diagnostic performance than determination of histamine release [21].

Investigation of drug allergies may be facilitated by the CAST® ELISA method, although, to date, there are few published studies. Kubota *et al.* [22] have reported that the CAST® ELISA test may be useful for investigation of patients with hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs). Identification of aspirin-intolerant patients using acetylsalicylic acid (ASA) and C5a-dependent sulphidoleukotriene release has been reported by Czech *et al.* [23]. According to May *et al.* [24], improved diagnostic sensitivity may be achieved when samples are stimulated with NSAIDs as well as ASA and C5a. Interestingly the method of Abrahamsen *et al.*

[11] did not distinguish aspirin sensitive asthmatics from non-allergic asthmatics without aspirin sensitivity.

Flow cytometric assay for determination of CD63 expression

The BASOTEST[®] manufactured by ORPEGEN Pharma (<http://www.orpegen.com>) relies upon the detection of CD63 on stimulated basophils as an indicator of allergen-induced activation. Heparinized blood samples (100 µL per test) are stimulated with allergen extracts prior to labelling with dual colour antibody reagents (anti-IgE phycoerythrin; anti-CD63 FITC). Following erythrocyte lysis, the percentage of activated basophils (IgE⁺/CD63⁺) is determined by flow cytometric analysis. Values of > 15% positivity are purportedly indicative of an individual being allergic to the allergen under test. The kit, as supplied, contains mixed grass and mite extracts as test reagents and is marketed for *in vitro* diagnostic use.

The assay has the potential to accommodate the use of a range of allergenic stimuli. In one such study, Pâris-Köhler et al. [25] have recently reported that the BASOTEST[®] is an effective diagnostic test for cypress pollen allergy, with comparable assay specificity and superior sensitivity to specific IgE analysis by the CAP system. However, no significant correlations between *in vitro* (BASOTEST[®] and specific IgE) and *in vivo* investigations (SPT and nasal challenge) were observed in their group of patients.

The BASOTEST[®] may provide the clinical diagnostic laboratory with a novel approach to allergy diagnosis. However, comprehensive clinical investigations are required to validate the assay.

Conclusions

The role of basophil activation tests in the diagnosis of allergy and asthma remains to be determined. For such assays to enter the diagnostic repertoire a number of criteria must be fulfilled.

- The tests must offer good sensitivity, specificity, and positive and negative predictive values which have been established in well-conducted clinical trials which include an accepted 'gold standard' method. To date, there are relatively few such reports on basophil activation assays. Furthermore, the lack of universally agreed gold standards for allergy and asthma diagnosis will hamper such evaluations. However, the availability of commercial assays, such as those described above, which incorporate standardized reagents and protocols should enable such assay validation to be more readily undertaken.
- The tests must be reproducible and technically straightforward. The use of whole blood assays and automated methods should make the performance of basophil activation

assays easier and improve quality control. Furthermore, the fact that assays can be performed on 24-hour-old blood samples is advantageous to the clinical diagnostic laboratory.

- The tests must be cost-effective. In terms of diagnostic testing, basophil activation assays are relatively expensive. Thus, true diagnostic efficiency must be established with proven cost-benefit before these tests enter mainstream use. It is likely, however, that these assays will be performed only in specialized or regional laboratories and that their use will be limited to selective allergens for which other well-established assays are unavailable, or in situations where *in vivo* testing is contra-indicated.

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