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Allergen-induced mediator release tests

The diagnosis of allergic reactions in clinical practice is based on both clinical history and the determination of specific immunoglobulin E (IgE), either in the serum or on skin mast cells. However, for various reasons, identification of the causative factors is not possible in all the cases. Moreover, not all allergies are IgE-dependent. In an attempt to find sensitive, specific and cost-effective methods to investigate hypersensitivity reactions, *in vitro* tests were developed at a very early stage. Allergen-induced mediator release assays analyze the mediator released from effector cells, mainly peripheral blood cells, when stimulated *in vitro* with serial dilutions of the putative allergens. Described initially as research tools, they could well become diagnostic tests. However, relatively few high quality reports have been published so far. In this review, we will detail allergen-dependent histamine, tryptase, arachidonic acid metabolite, e.g. cysteinyl leukotrienes and 15-hydroxyeicosatetraenoic mediator release tests.

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The diagnosis of allergy is easy in clinical practice and is based on a suggestive history, on positive skin tests and/or on specific immunoglobulin E (IgE) to the relevant allergens. Skin prick tests represent the major tool for the diagnosis of IgE-mediated diseases (1, 2). However, not all allergen extracts are available and not all allergies are IgE-dependent. Moreover, many hypersensitivities (i.e. clinical reactions resembling allergy) do not involve immune mediated processes (3) and identification of the precipitating factors is mandatory to enable allergen avoidance and to prevent a worsening of the disease which includes life-threatening reactions. Drug allergy and occupational allergy diagnosis, for example, are particularly difficult (4, 5) and currently set on clinical history and skin testing for a few allergens only. Controlled challenges in these cases certainly have the best sensitivity but are potentially harmful. They can be performed only in specialized centres, under the most rigorous surveillance conditions because of potential life-threatening reactions.

Therefore, *in vitro* tests are of tremendous interest to allergists in order to establish the culprit agent and to avoid provocations, especially for patients having contact with multiple allergens simultaneously and obviously for nonallergist physicians. These biological tests include serum allergen-specific antibodies (6–8), histamine, tryptase and other mediator measurements in body fluids (9–11), cellular membrane activation markers including the CD45 and CD63 basophil markers (12, 13), T-cell based assays (proliferation, activation, clones) (14) and mediator release tests (15). The measurement of mediators released in peripheral blood, nasal and bronchial secretions, sputum, skin blisters or in the urine during an allergic reaction was made possible by the development of highly specific and sensitive immunoassays for the titration of histamine, tryptase, prostaglandin (PG)D2, 15-hydroxyeicosatetraenoic (HETE), cysteinyl leukotrienes (CysLT) and eosinophil cationic protein (ECP) as best examples. They can be measured at baseline and after *in vivo* allergen challenge and provide important research tools. However, there is little application to the diagnosis of allergy in routine for these tests.

Allergen-induced mediator release tests explore the ability of effector cells to generate *in vitro* the mediators cited above in the presence of increasing doses of allergens. Described initially as research tools (15, 16), they are not widely utilized as routine biological assays because they sometimes require certain skills, they are time-consuming and expensive, they are seldom standardized and validated in clinical practice and because data concerning the assay performance characteristics are poor. However, they could well become diagnostic tests, especially with the availability of commercial assays (Table 1). In this review, we will detail histamine, tryptase, arachidonic acid metabolite, e.g. Cys-LT and 15-HETE mediator release tests.

Histamine release test

Utility as a research tool

The cross-linking of IgE on basophils is known to cause the release of many mediators including histamine, which can however also be liberated by non-IgE dependent mechanisms (17). In order to demonstrate

Table 1. Mediator release assays available commercially

Company	Web site address	Method	Mediator	Allergens provided
Hycor Biomedical Inc.	http://www.hycorbiomedical.com	ELISA	Histamine	YES
IBL GmbH	http://www.ibl-hamburg.com	ELISA	Histamine	NO
Immunotech	http://www.coulter.com	RIA	Histamine	
		ELISA		NO
Reflab	http://www.reflab.dk	Fluorimetry	Histamine	YES
Pharmacia & Upjohn	http://www.diagnostics.nu	FEIA	Tryptase	NO
Bülhmann Laboratories	http://www.bulhmannlabs.ch	ELISA	LTC4-LTD4-LTE4	YES

ELISA, enzyme-linked immunosorbentassay; FEIA, fluoroenzyme Immunoassay; RIA, radioimmunoassay.

allergen-induced histamine release, researchers developed this *in vitro* assay in the 1960s (16). In that paper, ragweed induced-basophil histamine release was better correlated to the clinical severity of rhinitis than antibody measurements. However, some ragweed allergic patients (approximately 10%) have nonresponding basophils (18). Passive sensitization of donor basophils with plasma samples from allergic patients, followed by their *in vitro* stimulation with the relevant aeroallergen, appeared to be slightly better correlated to radio-allergen sorbent test (RAST), skin tests and bronchoprovocation than direct histamine release (19). In the case of allergy to myorelaxants, the release of histamine correlates well with skin tests and specific IgE (17, 20).

Description of the assay

Histamine release is usually measured after venous blood is drawn into heparinized tubes and away from skin testing and provocation. The *in vitro* challenge is carried out at 37°C using diluted whole blood as well as increasing concentrations of both anti-IgE (from 0 to 10 μg/ml, to assess the spontaneous histamine release) and allergen. Supernatants, containing the histamine released, are then collected and frozen at −20°C until histamine measurements are performed. The total histamine content of the blood cells is measured after cell lysis. Histamine is titrated using fluorimetry or highly specific and sensitive radioimmunoassay (RIA) and enzyme-linked immunosorbentassay (ELISA). Assays are available commercially from different companies (Table 1) (15). Results are expressed as percentage of total histamine content after correction for spontaneous release. The net histamine release is calculated as follows: (anti-IgE release or allergen release – spontaneous release) / (total histamine content – spontaneous release). Many authors suggest 5% as the limit of positivity (20–24). Griese et al. (24) used 20% for house dust mites and 5% for fongi. Maly et al. (25) determined the cut-off values as the mean + 3 SD of the results obtained from 10 nonallergic controls. They varied from 1 to 20% according to the bee or wasp venom concentrations used. Receiver operating characteristic curves have rarely been employed to determine the appropriate cut-off value.

Utility as a diagnostic test

For house dust mite allergy, in 69 children diagnosed by bronchoprovocation (Table 2), Griese et al. (24) found that the histamine release test had a better sensitivity (87%) than both skin tests (66%) and RAST (76%). The sensitivity was also high (94%) in Ostergaard et al. study (26). For food allergy, in adults allergic to egg and/or milk diagnosed by double-blind placebo controlled challenge, Norgaard et al. (27) found that the histamine release test with fresh food allergens had a lower sensitivity (89%) than skin tests (100%). Using commercial food allergens, the sensitivity was only 56% (as compared with 75% for skin tests). Similarly and using a commercialized automated system, Li et al. (28) found comparable results with a sensitivity of 53% (as compared with 82% for skin tests and specific IgE). For hymenoptera venom allergy, Maly et al. (25) found in 11 honey bee- and 16 wasp-allergic patients, diagnosed by skin tests and specific IgE, that the sensitivity was 64% for bee venom allergy and 50% for yellow jacket venom allergy. For latex allergy, the sensitivity of the histamine release test reported in the Marais et al. study (28) is far below (45%) the one known for skin tests and specific IgE measurements. For drug allergy, although there have been numerous case reports in the literature showing some basophil histamine release, the utility of this methodology is globally not adequate, with a positive predictive value of 29% and a negative predictive value of 81% (23). Obvious reasons for low sensitivity – only 51% in our study (23) – are that the reactive compounds may not be the drug itself but one of its metabolites and/or that the soluble form of the drug may not be available (ready to be tested on blood basophils) and/or that the mechanism is not IgE-dependent. The low specificity of the histamine release test in the diagnosis of drug allergy – 63% in our study (23) may be related to the fact that, as for specific serum IgE, positive results demonstrate sensitization to the drug but do not confirm its responsibility in the allergic reaction. There may however exist subgroups of drug allergic patients for which histamine release may be of interest. In the case of myorelaxant allergy for example, Mata et al. (20) found a sensitivity and specificity of 65 and 100% respectively.

Table 2. Sensitivity and specificity of allergen-induced mediator release tests for the diagnosis of allergy (a selection of published data with more than 15 subjects tested)

				Allergic patientss		Non-allergic subjects	
Author (ref)	Year	Gold standard	Allergen tested *in vitro*	Se (%)	n	Sp (%)	n
Histamine release							
Griese et al. (24)	1990	CH + PT	HDM	87	38	84	31
			Mold	86	14	90	10
Ostergaard et al. (26)	1990	CH + PT	HDM Cat, dog	94	47	78	32
Norgaard et al. (27)	1992	CH + PT	Commercial food	56	9	78	9
			Fresh food	89	9	78	9
Li et al. (28)	2000	CH + ST + PT	Food	53	NA	82	NA
Marais et al. (29)	1997	CH	Latex	45	33	NA	NA
Maly et al. (25)	1997	CH + ST + IgE	Honey bee	64	11	50	12
			Yellow jacket	50	16	43	7
Demoly et al. (23)	1999	CH + ST ± PT	Drugs	51	35	63	73
Lebel et al. (39)	2001	CH + ST ± PT	ß-lactams	53	30	53	33
			Aspirin	55	19	35	17
Tryptase release							
Monasterolo et al. (32)	1997	CH + ST + IgE	Allergen	72	298	57	150
Rossi et al. (34)	1998	CH + ST + IgE	HDM	64	247	78	137
CysLT release							
Rossi et al. (34)	1998	CH + ST + IgE	HDM	71	247	87	137
Moneret-Vautrin et al. (38)	1999	CH + ST ± PT	Food	85	13	100	18
Marais et al. (29)	1997	CH	Latex	43	33	NA	NA
Maly et al. (25)	1997	CH + ST + IgE	Honey bee	82	11	67	12
			Yellow jacket	81	16	100	7
Kubota et al. (42)	1997	CH	NSAIDs	68	25	90	10
May et al. (43)	1999	CH + PT	Aspirin	18	38	99	50
			Aspirin + C5a				
				50	38	99	50
Lebel et al. (39)	2001	CH + ST ± PT	ß-lactams	43	30	79	33
			Aspirin	21	19	88	17
15-HETE release							
Kowalski et al. (50)	2002	CH + PT	aspirin	80	35	80	31

CH, clinical history; HDM, house dust mites; NA, not available; NSAIDs, nonsteroidal anti-inflammatory drugs; PT, provocation test; ST, skin tests; 15-HETE, 15-hydroxyeicosatetraenoic.

Tryptase assay

Utility as a research tool

Mast cells and basophils can be discriminated by their granule or secretory products; mast cells, in contradistinction to basophils release high quantities of tryptase and PGD2. Tryptase is present in very small amounts in basophils ($<1\%$ of those in mast cells) (30). Its release parallels histamine release. Because of its stability and in contrast to histamine, its immunoreactivity is not lost in the serum for several hours. Because it is not present in other cells, tryptase level in the serum or tissue fluids is a clinical indicator of mast cell activation in diseases such as systemic anaphylaxis and systemic mastocytosis (11, 31).

Description of the assay

As for histamine release from cell supernatants, tryptase release can be measured after venous blood is drawn into tubes containing heparine or ethylenediaminetetraacetic acid and *in vitro* challenge is performed using increasing concentrations of allergen or proper negative and positive controls [for 20 h at $+4°C$ in Monasterolo et al. study (32)]. Tryptase is titrated using RIA (32–34) or the commercialy available UniCAP-Tryptase fluoroimmunoassay (31) (Pharmacia Diagnostics AB, Uppsala, Sweden). Levels of tryptase under 1 μg/l are undetectable.

Utility as a diagnostic test

Two studies (32, 34) from the same group have evaluated basophil tryptase after allergen challenge *in vitro* (Table 2). In the first study (32), in 298 patients suffering from allergy (no details given), not only sensitivity (72%) and specificity (57%) were weak but also there was no correlation between tryptase levels and basophil numbers and serum IgE concentrations. In the second study (34), in 247 patients allergic to house dust mites, the sensitivity (64%) of the assay was lower and specificity better (78%), but still not clinically useful.

Cysteinyl leukotriene assay

Utility as a research tool

The cross-linking of IgE on basophils is known to cause the release of both histamine and CysLT, particularly when pretreated with the cytokines interleukin-3 (IL-3), IL-5 or granulocyte macrophage-colony stimulating factor (GM-CSF) (35). Increased amounts of Cys-LT have been found in nasal and bronchial lavages after natural and experimental allergen exposure (36). In order to demonstrate that allergen also induced Cys-LT release after *in vitro* challenges, researchers have more rapidly developed and commercialized this assay (37).

Description of the assay

The release of Cys-LT produced *in vitro* by isolated peripheral blood leukocytes after allergen stimulation is measured according to the Cellular Antigen Stimulation Test (CAST® ELISA) (37), commercialized by Bühlmann Laboratories AG, Allschwil, Switzerland (Table 1). Venous blood is drawn into heparinized tubes. Blood specimens are stored at 20–25°C for 2–3 h before leukocytes are isolated by dextran sedimentation in a polypropylene tube. *In vitro* stimulations are carried out using cell suspension and stimulation buffer (spontaneous release), or positive control (monoclonal antibody directed against the high affinity IgE receptor FcεRIα) or increasing concentrations of allergen in the presence of IL-3. After incubation at 37°C, cells are centrifuged and supernatants are collected and frozen at −20°C until measurements are performed. Cysteinyl leukotrienes (LTC4/D4/E4) are measured using the ELISA technique provided in the CAST® ELISA kit. This assay has recently been superseded by the CAST® 2000 ELISA.

Utility as a diagnostic test

For house dust mite allergy, in 247 patients diagnosed by clinical history, skin tests and specific serum IgE (Table 2), Rossi et al. (34) found that the CAST assay had a positive predictive value of 88%. For hymenoptera venom allergy, Maly et al. (25) found in 23 patients allergic to bee and/or wasp venoms, that the sensitivity was 81% for yellow jacket venom allergy and 82% for honey bee venom allergy. For food allergy, in the Moneret-Vautrin et al. study (38), the performance of the test was good with a sensitivity of 85% and a specificity of 100%. For latex allergy, the sensitivity of the CAST assay did not provide better results than the histamine release test in the study reported by Marais et al. (29). Various *in vitro* cellular tests have been described over recent years for the difficult diagnosis of drug allergy, and most data on the CAST assay exist for ß-lactams and nonsteroidal anti-inflammatory drugs (39–43). Conflicting results have emerged for immediate hypersensitivity reactions to ß-lactams with sensitivity of the assay ranging from 35 to 80% and specificity from 79 to 89%. For immediate hypersensitivity reaction to nonsteroidal anti-inflammatory drugs, the sensitivity range was worse (18–68%) and specificity better (79–100%). The low sensitivity of the CAST assay, as for the histamine release test, may come from the fact that either the reactive compounds may not be the drug itself but one of its metabolites, or the injectable soluble form of the drug may not be available (ready to be tested on blood basophils), and/or the mechanism is not dependent on these mediators (or on the cells releasing these mediators). Another obvious reason for low sensitivity is the fact that the hypersensitivity may decrease and disappear over time. However, taken together, the current data demonstrate that the CAST® ELISA is not efficient enough for the diagnosis of immediate hypersensitivity reactions to ß-lactams or nonsteroidal anti-inflammatory drugs. Optimizing the CAST and/or focusing on some patients only may make this test valuable. Improvement of the CAST has already been proposed in some research laboratories (25, 43) and the CAST® 2000 ELISA is available. Using preincubation with C5a and aspirin, it was found that the sensitivity of the CAST for the diagnosis of aspirin sensitivity was higher (Table 2) when using aspirin alone (43).

15-HETE assay

Utility as a research tool

Campbell et al. (44) demonstrated that epithelial cells obtained from asthmatics by bronchial brushings released more 15-HETE, fibronectin and PGE2 (after stimulation with the calcium ionophore A23187) than those obtained from normal subjects. Epithelial cells cultivated from nasal polyps obtained from patients with and without aspirin hypersensitivity secrete the same basal and A23187-enhanced 15-HETE quantities. However, incubation of the cells with aspirin increases 15-HETE production in aspirin-sensitive patients only (45). Taken together, these studies, along with others after *in vivo* challenge (46), suggest that 15-HETE is a potential mediator of asthmatic and inflammatory reactions in the airways. The major source of 15-HETE in the airways is the 15-lipoxygenase enzyme (47). However, there are two 15-HETE enantiomers, arising from two different enzymes: 15(S)-HETE which is a 15-lipoxygenase product and 15(R)-HETE which is a cyclooxygenase-2 (Cox-2) product with an unknown function, occurring mainly when Cox-2 is acetylated by aspirin at Ser 516 position (48, 49).

Description of the assay

Peripheral blood leukocytes (50) are incubated with increasing doses of lysine-acetylsalicylic acid and 15-HETE is measured in cell supernatants by either

ELISA (50) or RIA (45). The sensitivity of the latter assay is 2.5 pg/100 μl. There is no assay commercialized as yet.

Utility as a diagnostic test

As 15-HETE generation upon aspirin challenge appeared to be specific to aspirin sensitive patients in the latter results (45), the authors designed a diagnostic test based on that background (50). When applied to peripheral mononuclear cells of 35 aspirin-sensitive patients with asthma and rhinosinusitis and of 31 aspirin-tolerant asthmatics, the authors found a sensitivity of 80% and a specificity of 80% (Table 2).

Conclusion

Identification of the causative factors of hypersensitivity reactions by currently available allergen-induced mediator release tests is not fully satisfactory, because of the multiple existing allergens, to the fact that not all allergies are IgE-dependent and to the relatively few high quality reports published so far. In order to find sensitive, specific, reproducible, robust, easy to use and cost-effective methods to investigate hypersensitivity reactions in clinical settings, these *in vitro* tests have to be further developed and validated in a sufficient number of patients and against an accepted gold standard method. The use of whole blood and automated commercially available methods should definitely be preferred. Noteworthy, the introduction of flow cytometric assay for the determination of allergen-induced basophil activation membrane markers such as the CD63 has to be followed carefully because, once fully validated, this quick (results are available within 3 h) and less expensive methodology could be rapidly spread because of the numerous bench top flow cytometers already set in specialized laboratories (15).

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