

Diagnostic Tests Based on Human Basophils: More Potentials and Perspectives than Pitfalls

A.L. de Weck^a M.L. Sanz^a P.M. Gamboa^b W. Aberer^d J. Bienvenu^e
M. Blanca^c P. Demoly^f D.G. Ebo^h L. Mayorga^c G. Monneret^e J. Sainte-Laudy^g

^aUniversity of Navarra, Pamplona, ^bHopital Basurto, Bilbao, and ^cCarlos Haya Hospital, Malaga, Spain;

^dUniversity of Graz, Graz, Austria; Centres Hospitaliers Universitaires, ^eLyon, ^fMontpellier and ^gLimoges, France;

^hDepartment of Immunology, University of Antwerp, Antwerp, Belgium

Key Words

Basophil activation • Drug allergy • Flow cytometry • Food allergy • Insect venoms • β -Lactams • Latex • Myorelaxants • NSAID

Abstract

For the diagnosis of allergy, cellular basophil activation tests (BAT), e.g. histamine or sulfidoleukotriene release tests, have long been introduced, but the expression of basophil activation markers such as CD63 and CD203c detected by flow cytometry has attracted more recent attention. A recent opinion paper in this Journal has stressed not only the potential but also the possible pitfalls of flow-cytometric BAT. We have applied clinical validation of various BAT in various ways for several years, and our experience shows that these new technologies have more potentials and perspectives than pitfalls. A comprehensive review of clinically validated studies on allergy to aeroallergens, insect venoms, latex, food allergens and drugs, e.g. myorelaxants, β -lactams, pyrazolones and non-steroidal anti-inflammatory drugs, as well as chronic urticaria shows clearly that even with different protocols, reproducible and meaningful results can be obtained. Although the available technologies may still be optimized and better standardized, there are no serious rea-

sons to deprive allergic patients of clinically indicated BAT, which can be performed reliably by any laboratory with allergy and flow-cytometric capacity and expertise.

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Introduction

In a recent issue of this Journal an opinion paper was devoted to diagnostic tests in allergy based on human basophils [1]. Although reviewing first many publications about histamine release as an outcome of basophil activation, the main discussion and criticisms of this paper focus on the flow-cytometric basophil activation test (BAT), which has recently attracted increased attention.

The overall picture is strongly influenced by the experience gained with anti-IgE- and allergen-induced histamine release. In a number of ways, expression of basophil activation markers, particularly when applied to diagnostic clinical evaluation of basophil activation, obeys different rules, and a number of pitfalls are simply counteracted by our long clinical experience with such tests. The above-mentioned opinion paper [1] also nourishes the general impression that the diagnostic use of flow-cytometric BAT is plagued with many unresolved ques-

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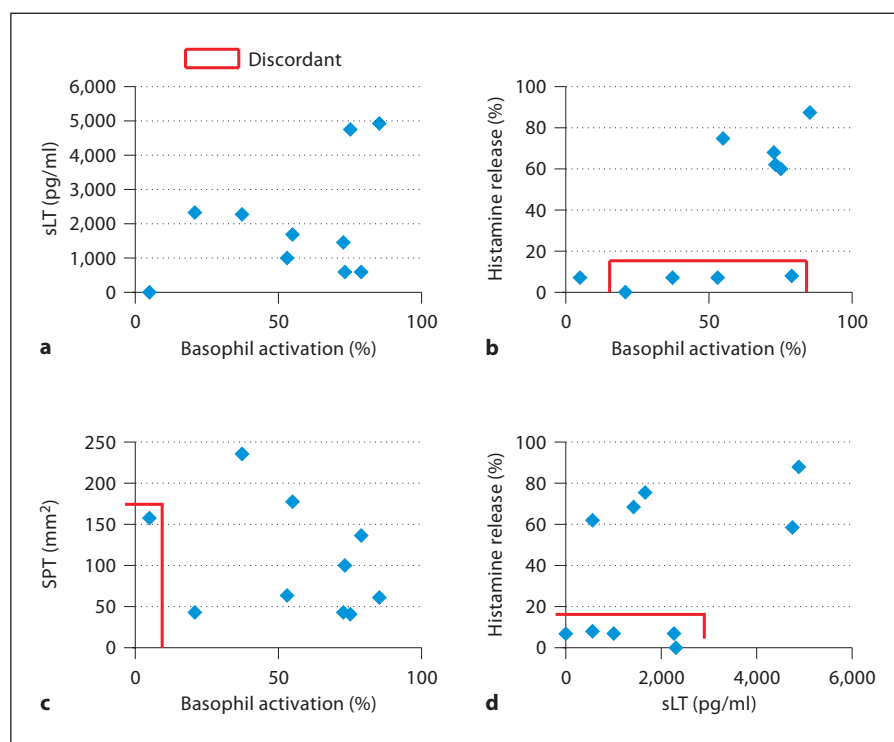
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Correspondence to: Prof. Alain de Weck
Beaumont 18
CH–1700 Fribourg (Switzerland)
Tel. +41 26 424 6910, Fax +41 26 424 6911
E-Mail alain.dew@bluewin.ch

Fig. 1. Dissociation of basophil activation outcomes in some individuals. Despite overall correlation between skin prick tests, sLT (CAST), histamine release and basophil activation (CD63; % = % of activated cells), individual patients may show total dissociation between these outcomes. Data for peach-allergic patients and rPru p 3 allergen [data from ref. 71]. nPru p 3: correlation between BAT and CAST (**a**; $r = 0.39$); BAT and the histamine release test (**b**; $r = 0.66$); BAT and the skin prick test (SPT; **c**; $r = -0.35$), and CAST and the histamine release test (**d**; $r = 0.49$).



tions and that it should be reserved for the time being to basophil-experienced laboratories.

In our opinion, some points need to be readdressed to complete the discussion. First, the clinical issues are discussed in a comprehensive review. More technical issues and controversies are addressed in a following paper [2]. Readers specifically interested in flow-cytometric BAT are also referred to some recent reviews [3–10].

Mechanisms of Basophil Activation

The mechanisms of IgE-receptor-mediated activation and the subsequent transmission of signals leading to the release of various mediators such as histamine, LTC₄ (sLT) and lymphokines, e.g. interleukin (IL)-4 and IL-13, have been well described [1] (fig. 1). However, the expression mechanisms of various membrane proteins, which are the basis of diagnostic BAT, as well as the various mechanisms of non-IgE-mediated activation have not yet been differentiated, leading to the impression that the same quantitative and qualitative rules may apply to the various outcomes of basophil activation (e.g. CD63/CD203c expression, histamine release, LTC₄ and lymphokine formation). Indeed, the authors [1] state that ‘the

parameters comprehensively studied during histamine release form the basis of the test outcome, independently of the final test readout. Promoting cellular tests for the evaluation of IgE-mediated sensitization has the caveat of introducing a number of interrelated variables’. As a matter of fact, and as discussed in greater detail elsewhere [2], the various test readouts do not strictly follow the rules established for histamine release: a number of differences in their activation cascades and regulation have been well documented [11–19].

These differences probably explain why in clinical practice, although often correlated, the various outcomes may occur independently from each other in some individuals (fig. 1).

For example, in a number of individuals and according to the mode of activation, expression of CD63, LTC₄ production and histamine release may be entirely dissociated for the same allergen concentration (fig. 2). In addition, for in vitro IgE-mediated reactions, the expression of CD63 is much more sensitive to the external Ca²⁺ concentration than LTC₄ production, and this relative Ca²⁺ sensitivity varies from one individual to another. This has led in some circumstances to the paradoxical finding of a control negative BAT but positive sLT (CAST assay) [Sanz, unpubl. data]. Even the expression of two different

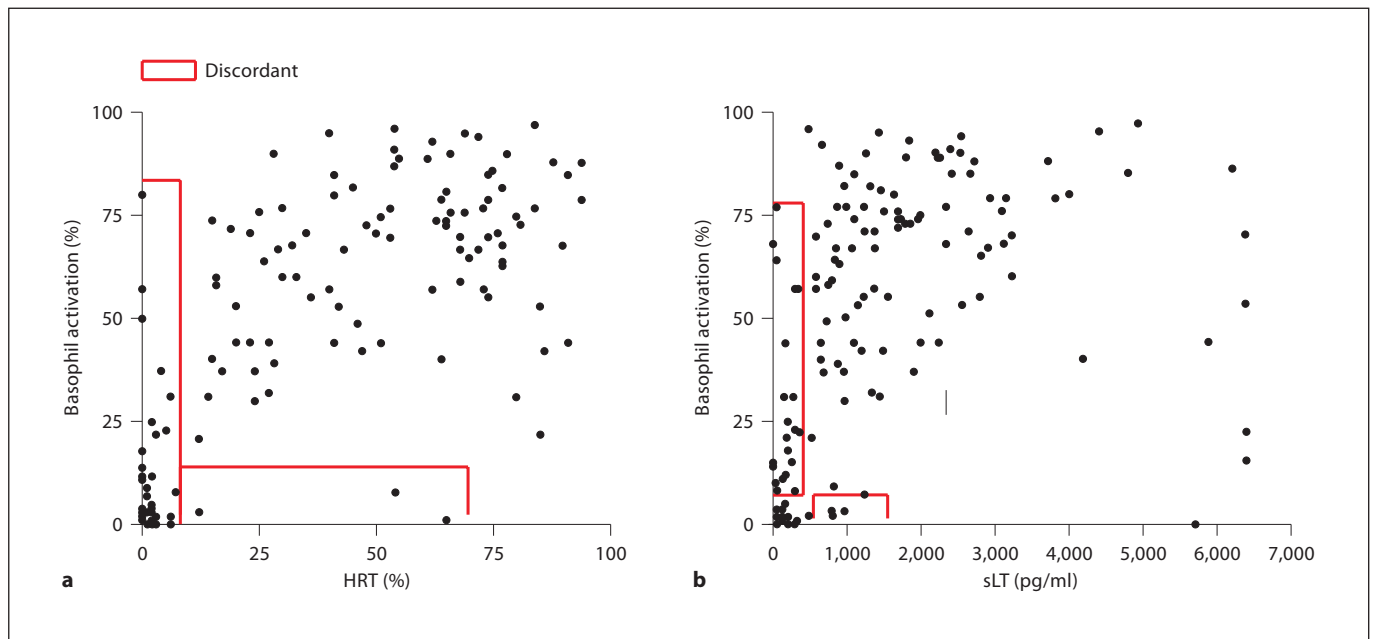


Fig. 2. Dissociation between CD63 expression, histamine release and LTC₄ production. Despite strong overall correlation between CD63 expression, histamine release and sLT production (CAST), a sizeable number of patients show total dissociation between these outcomes. Data from patients allergic to aeroallergens (house dust mite d 1 and *Lolium perenne*) [data from ref. 26]. HRT = Histamine release test. **a** $r = 0.79$, $p < 0.001$, $n = 144$. **b** $r = 0.7$, $p < 0.001$, $n = 180$.

membrane markers, CD63 and CD203c, obeys different rules and regulations [20, 21].

One must therefore be careful when extrapolating general conclusions drawn from experience gained with histamine release to other BAT, a point more extensively discussed elsewhere [2].

Clinical Issues in the Diagnostic Use of BAT

In addition to the large number of anecdotal studies at the beginning of the BAT era in the 1990s [22–24], during the past 5 years, a large number of clinically validated studies have been published in peer-reviewed and highly ranked journals that focus on clinical allergy. Most of these publications emerged from European groups. Clinical studies encompassing at least 15–20 confirmed allergic patients were considered validated, demonstrated by compelling history and/or provocation tests as well as other positive skin tests and/or allergen-specific IgE for example. In addition, at least 10, non-allergic controls negative to skin tests and other allergy diagnostic tests with no history of aeroallergen sensitization should be

included. For some allergens, e.g. food, enrolment of atopic patients is required, i.e. patients with IgE to aeroallergens but negative to challenge with the food allergen tested. Many of these studies have been reviewed recently [8–10]. Herewith, tables 1–7 summarize the results of validated clinical studies performed with various allergens such as inhalant allergens [25–33] (table 1), hymenoptera venoms [34–56] (table 2), latex [57–63] (table 3), food [64–80] (table 4) and drugs [81–125] (tables 5–7). The techniques applied are also indicated. In our opinion, these studies demonstrate the diagnostic utility of BAT for a number of clinical indications.

Since some specific questions or criticisms have been raised [1] about some of these studies, we feel it is appropriate to address them in more detail.

Clinical Indications of BAT

Hymenoptera Allergy

The utility of BAT in hymenoptera venom hypersensitivity [34–56] has been confirmed hitherto by many more than the two studies quoted by Kleine-Tebbe et al.

Table 1. Clinically validated studies for aeroallergens

Ref.	Authors, year	Allergens	Patients	n	Controls	n	Sens., %	Spec., %	Cells	CD	IL-3	Test
25	Cozon et al., 1999	mites d1	SPT+	10	HC	NA	78	91	Wb	63	+/-	
25	Cozon et al., 1999	grass pollen	SPT+	13	HC	NA	100	100	Wb	63	+/-	
27	Paris-Kohler et al., 2000	cypress pollen	SPT+	34	HC	33	91.2	100	Wb	63	+	
26	Sanz et al., 2001	mites d1	SPT+	58	HC	38	91.3	98.4	Il	63	+	
26	Sanz et al., 2001	<i>Lolium perenne</i>	SPT+	51	HC	38	91.3	98.4	Il	63	+	
29	Hauswirth et al., 2002	Bet v1, 2 Phl p 1, 3, 5	SPT+	20	HC	10	100	100	Wb	203c	-	
30	Michova et al., 2006	grass pollen	SPT+	56	HC	14	85	100	Wb	63	+	Baso
	Michova et al., 2006	grass pollen	SPT+	56	HC	14	72	92	Wb	63	+	BD

Baso = BASOTEST; BD = FastImmune Becton-Dickinson; HC = healthy controls; Il = isolated leukocytes; NA = not available; Ref. = reference; Sens. = sensitivity; Spec. = specificity; SPT = skin prick test; Wb = whole blood. For listed studies, see the reference list. In addition, several other studies have been presented and published, sometimes in abstract form [28, 30–33].

Table 2. Clinically validated studies for insect venoms

Ref.	Authors, year	Allergens	Patients	n	Controls	n	Sens., %	Spec., %	Cells	CD	IL-3
34	Sabbah et al., 1998	hymenoptera	AS	44	HC	30	100	100	Wb	63	-
35	Sainte-Laudy et al., 2000	hymenoptera	AS	45	HC	32	93	100	Il	63	-
36	Freitag et al., 2001	wasp	AS	20	HC	12	70–100	100	Wb	63	+
37	Platz et al., 2001	hymenoptera	AS	22	HC	13	91		Wb	203c	-
38	Lambert et al., 2003	hymenoptera	AS	28	HC	8	91	90	Wb	63	-
39, 40	Erdmann et al., 2003, 2004	wasp	AS	50	HC	20	92	80	Wb	63	-
41	Sturm et al., 2004	hymenoptera	AS	57	HC	30	88	87	Wb	63	-
43	Eberlein-König et al., 2004	hymenoptera	AS	14	HC	5	78	100	Wb	63	+
44, 45	Scherer et al., 2005, submitted	wasp	AS	150	HC	40	93	85	Il	63	+
		bee	AS				94	95	Il	63	+
46	Eberlein-König et al., 2006	wasp, bee	AS	67	HC	10	97	100	Wb	63	+
47	Eberlein-König et al., 2006	wasp, bee	AS	43	HC	25	97	89	Wb	203c	-
							89	100	Wb	63	+
48	Ebo et al., 2007	wasp	AS	80	HC	14	86	100	Wb	63	+

AS = Anaphylactic shock; HC = healthy controls; Il = isolated leukocytes; n = number of patients; Ref. = reference; Sens. = sensitivity; Spec. = specificity; Wb = whole blood. [For additional studies on insect venoms, see ref. 49–56.]

[1] (table 2). The main benefit of BAT in that indication is that it may confirm clinical history in the absence of positive skin tests or positive allergen-specific IgE. In addition, by using BAT, the insect responsible for clinical sensitivity may be detected leading to a more specific indication for immunotherapy [43, 45]. There is no reason to cast doubt on the results, as possibly due to aggregated platelets [1], since specific investigations on that point [54, 126] have been negative. In the largest series reported so far [44, 45], correlation of BAT with skin tests and specific IgE ranged from 0.71 to 0.82. In a recent large series of 118 patients, a definite diagnosis of venom anaphylax-

is [history + unequivocal single positive specific IgE (sIgE) and skin test] was observed in only about 55% of the patients. In 40%, diagnosis is hindered by equivocal or discrepant test results (mostly double-positive sIgE results for both venoms). In 7 patients (5%), there was no proof of sensitization (sIgE and skin test negative). Considering only BAT responders (86% of all patients), BAT confirmed the final diagnosis in almost two thirds of the patients with equivocal test results and, most importantly, in all 7 patients with negative sIgE and skin test results [48]. This paper [48] has documented that BAT may also be used to monitor venom immunotherapy, although

Table 3. Clinically validated studies for latex allergens

Ref.	Authors, year	Allergens	Patients	n	Controls	n	Sens., %	Spec., %	Cells	CD	IL-3
57	Ebo et al., 2002	latex	Hx+ ST+ sIgE+ Hx+ ST- sIgE- Hx- ST- sIgE+	29 13 24	HC, STsIgE HC, STsIgE+	12 24	91.7 100 17	100 87 83	Wb	63	+
58	Sanz et al., 2003	latex	Hx+ ST+	43	HC, Prov.-	20	93	100	Il	63	+
59	Erdmann et al., 2003	latex	Hx+ ST+	20	HC5, AC5	10	90	80	Wb	63	+
60	Boumiza et al., 2003	latex	Hx+ ST+	16	HC	12	50 75	100 100	Wb Wb	63 203c	- -
61	Hemery et al., 2005	latex	Hx+ ST+ sIgE+	46	HC	33	85	97	Wb	63	+
62	Sanz et al., 2006	Hev b 5, 6	Hx+ ST+ sIgE+	23	HC	10	95	100	Il	63	+
63	Nettis et al., 2006	Hev b 5, 6	Hx+ ST+ IgE+	24	HC	19	96	100	NA	NA	NA

AC = Atopic controls; HC = healthy controls; Hx = history; Il = isolated leukocytes; NA = not available; Prov. = provocation; Sens. = sensitivity; sIgE = specific IgE; Spec. = specificity; ST = skin test; Wb = whole blood.

Table 4. Clinically validated studies for food allergens

Ref.	Authors, year	Allergens	Patients	n	Controls	n	Sens., %	Spec., %	Cells	CD	IL-3
64	Moneret-Vautrin et al., 1999	various	Prov.+	31	HC	24	80	100	Il	63	+
65	Garcia-Aviles et al., 2000	various	Hx+	74	AC	64	82.3	62.5	Il	63	+
66	Diaz-Perales et al., 2002	peach, rPru p 3	AS	17	HC	5	90	100	Il	63	+
67	Erdmann et al., 2003	carrot, celery, hazelnut,	OAS+	29	HC	20	85–90	80–90	Wb	63	+
68	Ebo et al., 2005	apple	OAS+ OAS-	29 23	HC	10	100 86	100 75	Wb	63	+
69	Erdmann et al., 2005	rMal d 1, rApi g 1, rDau c 1	OAS+ OAS-	32 12	HC	10	65–75	68–100	Wb	63	+
70	Caceres et al., 2005	rMal d 1, 3, 5; rPru p 3	AS	24	HC	8	67–92	83–100	Il	63	+
71	Gamboa et al., 2007	rMal d 1, 3, 4, rPru p 3	Prov.+	30	HC	29	~100	~100	Il	63	+

In addition to the listed studies, a number of reports on individual food-related allergens have been published [71–79]. AS = Anaphylactic shock. For further information, see legend to table 3.

Table 5. Clinically validated studies for myorelaxants

Ref.	Authors, year	Allergens	Patients	n	Controls	n	Sens., %	Spec., %	Cells	CD	IL-3
81	Leynadier et al., 1996	myorelaxants	AS	15	HC Prov.-	9	66	87	Wb	63	-
82	Abuaf et al., 1999	myorelaxants	AS, ST+	41	HC Prov.-	21	64	100	Wb	63	-
83	Monneret et al., 2002	myorelaxants	AS, ST+	39	HC Prov.-10, AC 7	17	54	100	Wb	63	-
84	Sudheer et al., 2005	myorelaxants	AS, ST±	21	HC	10	79 36	100 100	Wb Wb	63 203c	- -
85	Kvedariene et al., 2006	myorelaxants	AS, ST?	47	AC 40, HC 5	45	36 (86 ^a)	93	Wb	63	+
86	Ebo et al., 2006	rocuronium	AS, ST+	14	HC ST- Prov.-	8	91	100	Wb	63	+

AS = Anaphylactic shock. For further information, see legend to table 3.

^a In patients investigated within 3 years after the clinical reaction.

Table 6. Clinically validated studies for β -lactams

Ref.	Authors, year	Allergens	Patients	n	Controls	n	Sens., %	Spec., %	Cells	CD	IL-3
88	Sanz et al., 2002	β -lactams	IT, ST+	58	HC	30	50	93	II	63	+
89	De Weck et al., 2002										
90	Torres et al., 2004	β -lactams	IT, ST+	70	HC	40	48	91	Wb	63	+
91	Arnoux et al., 2004	β -lactams	IT	35	AC	40	9	100	Wb	63	+
92	Gamboa et al., 2004	β -lactams	IT, ST- Prov.+	23	HC	30	39	93	II	63	+
93	Kvedariene et al., 2005	β -lactams	IT	22	HC	29	34	83	Wb	63	+
94	Erdmann et al., 2005	β -lactams	IT	20		NA	35	NA	Wb	63	+
95	De Weck et al, ENDA, 2006	β -lactams	IT ST $\pm$	181	HC	80	46	93	II	63	+

An additional report has been published [96]. IT = Immediate-type reactions (anaphylactic shock, urticaria, angioedema). For further information, see legend to table 3.

Table 7. Clinically validated studies for hypersensitivity to NSAID

Ref.	Authors, year	Allergens	Patients	n	Controls	n	Sens., %	Spec., %	Cells	CD	IL-3
100	Sanz et al., 2005	NSAID	NSAID syndrome	60	HC Prov.-	30	74	92	II	63	+
93	Kvedariene et al., 2005	NSAID	NSAID syndrome	15	HC	10	25	80	Wb	63	+
94	Erdmann et al., 2005	NSAID	NSAID syndrome	20		NA	30	NA	Wb	63	+
102	De Weck et al., ENDA, 2006	NSAID	NSAID syndrome	140	HC Prov.-	163	76	92 ^a	II	63	+

^a With a particular method of basophil isolation (buffy coat). For further information, see legend to table 3.

some other reports are less positive [39, 40]. When BAT are performed in whole blood, the question remains whether the decrease in reactivity observed after immunotherapy is due to competing venom-specific serum IgG antibodies, as shown for histamine release [127], or whether a true decrease in cellular reactivity also occurs in parallel.

Latex Allergy

In several validated studies [57–63] (table 3), BAT was shown to demonstrate a sensitivity and specificity for CD63 expression of >90%, with one exception in which sensitivity was reported to be 75% for CD203c and 50% for the CD63 assay [60]. BAT is the test which reflects the most closely clinical provocation. In particular, it enables to sort out the clinically 'false-positive' cases, namely those with negative latex challenge but positive latex-specific IgE determinations due to cross-reactive carbohydrate determinants [57]. With other allergens, IgE to cross-reactive carbohydrate determinants seems to have little functional activity [128], too, and BAT should permit to sort out such cases.

Food Allergy

Food allergy will probably become one of the areas of allergy diagnosis in which BAT will give most useful services [64–80]. Although double-blind, placebo-controlled food challenge (DBPCFC) is constantly advocated as the gold standard, it is recognized that it is not flawless and that it is in practice less often used than publicly advocated. In numerous instances, it is not practicable or even not ethical (e.g. history of anaphylactic shock). The clinical picture of the oral allergy syndrome (OAS) after single-blind provocation is so obvious that the requirement for DBPCFC in that instance is questionable. There are several validated studies in the literature (table 4), some including recombinant food allergens [66, 69–71]. In birch-allergic patients with or without OAS demonstrated by DBPCFC, cellular tests, e.g. CAST [73] and BAT [68], have been found to be better predictors of provocation outcome than quantitative food-specific IgE. BAT may help to discriminate between relevant and irrelevant IgE to apple [68] and it has also shown its diagnostic usefulness in allergies to various foods [74–80].

Chronic 'Idiopathic' Urticaria

Numerous cases of chronic 'idiopathic' urticaria seem due to autoimmune phenomena, in particular to autoantibodies directed against IgE or its receptors (IgE-RI), leading to immediate skin test reactivity against autologous serum but also to basophil hyperreactivity. An increasing number of studies has confirmed the usefulness of BAT with donor basophils in the diagnosis and pathogenetic elucidation of chronic urticaria [129–138].

Drug Hypersensitivity

It is probably in the field of immediate-type allergic reactions to drugs that BAT has attracted the most interest in recent years, due to the fact that the performance of drug-specific IgE tests has been rather poor, especially in other drugs than β -lactam antibiotics, and since they are not available for many drugs. BAT can also detect immediate-type drug hypersensitivities due to non-IgE mechanisms.

Myorelaxants [81–87]. There are currently five validated studies (table 5) showing the utility of BAT in the diagnosis of immediate-type reactions to myorelaxants, with a sensitivity of 54–91% for CD63 expression and 36% for CD203c expression [84]. One may argue that sensitivity is too low to be really useful in routine diagnosis [83], but one must bear in mind that it is at least equal if not superior to that of skin tests and drug-specific IgE. On the other hand, BAT is highly specific. Accordingly, similar to many other tests in drug allergy, a positive test in an individual case is probably meaningful, while a negative test does not permit to exclude clinical drug allergy. Sensitivity apparently increases up to 80% when BAT is performed shortly after the clinical reaction [85]. BAT has proven to be complementary to skin tests in the evaluation of cross-reactivity between myorelaxants [86].

β -Lactams [88–96]. In quoting only one [88] of the several validated studies on BAT in the diagnosis of β -lactam allergy and in pretending that CD63 BAT was only performed in patients with positive skin tests to β -lactams, Kleine-Tebbe et al. [1] have presented an incomplete view of the literature. If the percentage of positive BAT is only about 50% of skin-test-positive cases [88–90], it is also positive in about 40% of the patients with entirely negative skin tests and a positive clinical history of an immediate-type systemic reaction confirmed by provocation [92], who constitute about 25% of all β -lactam-allergic patients. A lower sensitivity has sometimes been found for BAT in whole blood, but this is neither a consistent finding (table 6) nor one obtained by direct comparisons [124]. In studies comparing sensi-

tivity and specificity of BAT with those of sIgE to β -lactam in the same patients [87–89, 91, 92], sensitivity of BAT determined by basophil activation was apparently superior (5–10% or more). Sensitivity of β -lactam sIgE varied between 24 and 74% [86, 92, 93, 95] depending on the group of patients selected and the time elapsed since the clinical reaction. Recently, these results have been confirmed in a large multicentric study implicating 10 European groups, 181 β -lactam-allergic patients and 80 controls [95]. In that study, it was also shown that the combination with CAST increases sensitivity to 62%. In terms of diagnostic performance, the combined use of skin tests, sIgE and BAT increases sensitivity to 85% and the addition of CAST to 95%.

Pyrazolones. In IgE-mediated allergies to single analgesics, e.g. pyrazolones, metamizol has attracted particular interest [99]. These patients differ from those with the NSAID hypersensitivity syndrome: they react specifically to a single drug or to a group of drugs of similar immunochemical structure. In 26 patients reacting to metamizol, BAT was found positive in 42%. BAT was also highly specific (100%). In combination with skin tests, sensitivity increased to 69% and combined with CAST to 77%.

NSAID [100–107]. Since several authors have stated that blood leukocytes from patients with the NSAID hypersensitivity syndrome may produce sLTs, as detected by CAST [108], the dogma that no in vitro tests may be associated with that condition has been crumbling. The first studies on BAT and NSAID by Sanz et al. [100] and Gamboa et al. [101] have not been properly quoted and interpreted [1]. If among 60 patients, only 18 have been effectively challenged, the remainder (42 patients) had a documented history of at least two or more immediate reactions to at least two different NSAID. This is a rather strict clinical inclusion criterion. Furthermore, in an ensuing multicentric study [102] on two similar populations who have been challenged or not (but with at least two clinical events) and analyzed separately, the percentage of positive BAT was identical (about 75%). It has also been questioned [1] why a BAT test to some NSAID could be considered positive when the culprit drug itself would be negative. One should realize that the mechanism of BAT in the NSAID hypersensitivity syndrome has nothing to do with an immunological reaction and that the classical notions of specificity for culprit drug and cross-reactivity do not apply. In fact, BAT reactions in NSAID hypersensitivity are closely related to the pharmacological COX-1-inhibiting activity of NSAID and are strictly dose dependent [100–105]. The most likely and main mechanism of

basophil activation is the pharmacological abolition of prostaglandin E₂ synthesis, a natural inhibitor of basophil activation, partly responsible for homeostasis [103–105]. In vitro, diclofenac and naproxen have a much stronger pharmacological activity than aspirin and are more likely to cause a BAT reaction in vitro, even if aspirin had been responsible for the clinical reaction in vivo. These findings [103, 105] have recently been confirmed in a large multicentric study including 12 European groups, 150 NSAID-hypersensitive patients and 163 controls [102]. A major new finding is that some NSAID-tolerant individuals also react to higher concentrations of NSAID in BAT: the clinical NSAID hypersensitivity syndrome appears to be reflected in a shift of the BAT dose-response curve to NSAID [101–105]. It has also been shown that an index based on BAT reactions to two concentrations of aspirin, diclofenac and naproxen (ADN index) enables to distinguish NSAID-hypersensitive from NSAID-tolerant patients with 80% accuracy [102]. Provocation indeed remains the ultimate proof needed for validation, but by now the BAT and the ADN index seem to be a practical option in many cases, e.g. when provocation cannot be done. There is some evidence that the mode of cell preparation (e.g. whole blood, buffy coat or isolated plasma leukocytes) may play some role in BAT reactivity to NSAID [103, 105] (table 7).

In some cases of anaphylactic shock to aspirin, an IgE-related mechanism has been suggested as the cause of a positive BAT [106]. It has also recently been claimed that diclofenac causes microscopic basophil degranulation but no CD63 expression [107]. Since these BAT-negative results have been obtained with drug concentrations 8–30 times lower than those found required for a positive BAT [97, 99], these results should not be considered relevant.

Others

Individual cases of immediate-type allergy to a number of other drugs or materials used in diagnostic or therapeutic procedures have been reported in recent years: iodinated povidone [109], cyclosporine [110], heparin [111, 112], chlorhexidine [113, 114], hydroxyethyl starch [115], patent blue [116, 117], omeprazole [118], hyaluronidase [119], *Viscum album* [120], Gelofusine [121, 122], dexchlorpheniramine [123] or bovine serum albumin [124]. 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.

In view of all these facts, the conclusion that the role of BAT in the diagnosis of drug hypersensitivity still re-

mains to be elucidated [1], which has been drawn in some position papers [1, 139–141], no longer represents the state of the art.

Indications, Performance and Interpretation of BAT

BAT are not primary diagnostic measures; they are essentially complementary to skin tests and allergen-specific IgE determinations, particularly when these cannot be performed or have given equivocal/doubtful results in comparison with clinical history. They are particularly indicated in insect venom allergy, food allergy, latex allergy and the major immediate-type drug allergies. BAT appear to be useful for the study of cross-reactivity among various protein allergens and drugs and for monitoring immunotherapy with some allergens and particularly with anti-IgE antibodies [15, 125].

The proper performance of the test requires special attention to details, particularly regarding the storage of the blood samples, the preparation and quality of the reagents used and the flow-cytometric gating processes established. Most important is the quality and standardization of the allergens used, which must be submitted to special quality control measures for use in flow cytometry. If these flow-cytometric quality control measures are not met by commercially available allergens, the laboratory will have to perform them by itself. Matter of fact, experience with allergens is probably more important for quality BAT than experience with basophil handling, which is easily acquired by any flow-cytometric laboratory. Some of these technical details are discussed in the accompanying paper [2] and in various reviews [5, 8, 9].

In order to establish appropriate cutoff points for each allergen, it is necessary to have proper negative and positive controls as well as receiver-operating characteristic curves (ROC) establishing optimal sensitivity versus specificity [142]. In fact, the recommendation to determine cutoffs by this method [8] has been followed by the majority of groups having published BAT clinical studies for a number of years.

In the experience of the Pamplona group, for example, the following cutoff points offer the highest specificity and sensitivity values determined by ROC [4, 10]: for inhalant allergens >15%; food allergens >15%; latex >10%, hymenoptera venoms >10%; β -lactam antibiotics >5% and stimulation index (SI) >2; metamizol >5% and SI >5; aspirin and NSAIDs >5% and SI >2. For most other groups [8, 9], cutoff points have been very similar but usually not adding an SI as additional requirement. For

some special issues, e.g. NSAID, special criteria (e.g. the ADN index) [102] have to be applied.

It has been stated [1] that the quantitative degree of basophil activation, measured by the amount of histamine or sLT released or the quantitative expression of activation markers in BAT, has presumably no clinical diagnostic value. This statement needs some qualification. Some authors have indeed found a correlation between the degree of clinical sensitivity and the percentage of activated basophils [50], but other authors did not confirm this observation. In a recent paper [54], it has been claimed that patients at risk of anaphylaxis upon insect sting challenge may show higher BAT baseline levels, but this study used different technologies and mode of evaluation than most BAT studies reported hitherto. For muscle relaxants, a correlation between the degree of skin sensitivity and CD63 expression has been reported [82]. The same has been the case for the amount of sLT released (CAST) and nasal allergen provocation [143]. In hymenoptera venom allergy, the degree of BAT reactivity has been reported to be predictive of side effects during venom immunotherapy [50, 51], but this is not agreed upon by all [52]. The occurrence of high CAST and/or BAT levels to β -lactams has been correlated to systemic anaphylaxis [89]. Finally, it has been demonstrated that sLT levels (CAST) [73] and BAT [68] are highly predictive of DBP-CFC and of OAS in food allergy.

Conclusions

There should be little doubt for those following the literature that such tests are rapidly becoming an important addition to our allergy diagnostic capacities. However, the number of European groups possessing varied and sustained practical BAT experience in routine diag-

nosis is still rather limited. Furthermore, some defiance has been entertained for years by position papers often authored by scientists not yet using the method in their own laboratory and always requiring still 'more evaluation' [139, 140].

The most recent opinion paper [1] on this matter, to which we wish to contribute herewith with our BAT clinical experience, may give the impression of an 'extraordinary variability of BAT responses', of an avalanche of pitfalls and of a requirement to possess many skills before engaging in clinical BAT diagnosis, 'which should be restricted to selected cases and to experienced laboratories'. It would be a pity if due to these warnings many patients would still be deprived of the benefits of BAT and flow-assisted allergy diagnosis.

In fact, the common positive scientific and clinical experiences with BAT gathered over a number of years are more valuable than the few technical issues still open to discussion, which are dealt with in detail elsewhere [2]. A special effort is under way to standardize procedures and evaluation at the European level [141]. In addition to a number of validated and published 'home protocols', we dispose at present of at least three widely clinically validated and commercially available BAT technologies (e.g. BASOTEST, Orpegen, Heidelberg, Germany; Fastimmune, Becton Dickinson, Mountain View, Calif., USA, and Flow Cast, Bühlmann Laboratories, Allschwil, Switzerland) [8, 10]. These may be considered as first-generation BAT. Modifications have been proposed (Allergenicity, Beckmann-Coulter, Marseille, France) [9] and additional second-generation tests still await an equivalent clinical validation. However, there are no rational or scientific reasons to temporize providing BAT services for proper clinical indications, in our opinion, for a clinical allergy group wishing to offer patients the benefit of optimal allergy diagnosis.

References

- 1 Kleine-Tebbe J, Erdmann S, Knol EF, Mac-Glashan DW Jr, Poulsen LK, Gibbs BF: Diagnostic tests based on human basophils: potentials, pitfalls and perspectives. *Int Arch Allergy Immunol* 2006;141:79–90.
- 2 De Weck AL, Sanz ML, Gamboa PM, Aberer W, Bienvenu J, Demoly P, Ebo DG, Mayorga L, Monneret G, Sainte Laudy J: Diagnostic tests based on human basophils: more potentials and perspectives than pitfalls. II. Technical issues. *J Invest Allergol Clin Immunol* 2008, in press.
- 3 Crockard AD, Ennis M: Laboratory-based allergy diagnosis: should we go with the flow? *Clin Exp Allergy* 2001;31:975–977.
- 4 Sanz ML, Maselli JP, Gamboa PM, Garcia-Aviles MC, Oehling A, Diéguez I, de Weck AL: Flow-cytometric basophil activation test. A review. *J Invest Allergol Clin Immunol* 2002;12:143–154.
- 5 De Weck AL, Sanz ML: Flow cytometric cellular allergen stimulation test (FAST/Flow CAST): technical and clinical evaluation of a new diagnostic test in allergy and pseudo-allergy. *Allergy Clin Immunol Int* 2002;14:204–215.
- 6 Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerk LD, Stevens WJ: In vitro allergy diagnosis: should we follow the flow? *Clin Exp Allergy* 2004;34:332–339.

- 7 Demoly P, Lebel B, Arnoux B: Allergen-induced mediator release tests. *Allergy* 2003; 58:553–558.
- 8 Ebo DG, Sainte-Laudy J, Bridts CH, Mertens CH, Hagendorens MM, Schuerwegh AJ, De Clerck LS, Stevens WJ: Flow-assisted allergy diagnosis: current application and future perspectives. *Allergy* 2006;61:1028–1039.
- 9 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;3:9–18.
- 10 Sanz ML, Gamboa PM, De Weck AL: In vitro tests: basophil activation tests; in Pichler W (ed): *Drug Hypersensitivity*. Basel, Karger, 2007, pp 389–400.
- 11 MacGlashan DW Jr, Peters SP, Warner J, Lichtenstein LM: Characteristics of human basophil sulfido-peptide leukotriene release: releasability defined as the ability of the basophil to respond to dimeric cross-links. *J Immunol* 1986;136:2231–2239.
- 12 MacGlashan DW Jr, Warner J: Stimulus-dependent leukotriene release from human basophils: a comparative study of C5a and Fmet-leu-phe. *J Leukoc Biol* 1991;49:20–40.
- 13 Miura K, Schroeder JT, Hubbard WC, MacGlashan DW Jr: Extracellular signal-regulated kinases regulate leukotriene C4 generation, but not histamine release or IL-4 production from human basophils. *J Immunol* 1999;162:4198–4206.
- 14 Miura K, Lavens-Philipps S, MacGlashan DW Jr: Localizing a control region in the pathway to leukotriene C₄ secretion following stimulation of human basophils with anti-IgE antibody. *J Immunol* 2001;167: 7027–7037.
- 15 MacGlashan DW Jr: IgE and FcεRI regulation. *Ann NY Acad Sci* 2005;1050:73–88.
- 16 Knol EF, Koendeman L, Mul FP, Verhoeven AJ, Roos D: Differential activation of human basophils by anti-IgE and formyl-methionyl-leucyl-phenylalanine. Indications for protein kinase C-dependent and independent activation pathways. *Eur J Immunol* 1991;21:881–885.
- 17 Dahinden CA, Kurimoto J, Baggiolini M, Dewald B, Walz A: Histamine and sulfido-leukotriene release from human basophils: Different effects of antigen, anti-IgE, C5a, f-Met-Leu-Phe and the novel neutrophil-activating peptide NAF. *Int Arch Allergy Appl Immunol* 1989;90:113–118.
- 18 Kurimoto Y, De Weck AL, Dahinden CA: The effect of interleukin 3 upon IgE-dependent and IgE-independent basophil degranulation and leukotriene generation. *Eur J Immunol* 1991;21:361–368.
- 19 MacGlashan DW Jr, Bochner BS, Adelman DC, Jardieu PM, Togias A, Lichtenstein LM: Serum IgE level drives basophil and mast cell IgE receptor display. *Int Arch Allergy Immunol* 1997;113:45–47.
- 20 Bühring HJ: The basophil-specific ectoenzyme E-NPP3 (CD 203c) as a marker for cell activation and allergy diagnosis. *Int Arch Allergy Immunol* 2004;133:317–329.
- 21 Hennersdorf F, Florian S, Jakob A, Baumgartner K, Sonneck K, Nordheim A, Biedermann T, Valent P, Bühring HJ: Identification of CD13, CD107a and CD164 as novel basophil-activation markers and dissection of two response patterns in time kinetics of IgE-dependent upregulation. *Cell Res* 2005;15: 325–335.
- 22 Sainte-Laudy J, Valon C, Guérin JC: Analyse de l'expression membranaire du marqueur CD63 par activation du basophile humain. Application au diagnostic allergologique. *Allerg Immunol (Paris)* 1994;26:211–214.
- 23 Sabbah A, Drouet M, Sainte-Laudy J, Lauret MG, Loiry M: Apport de la cytométrie en flux dans le diagnostic allergologique. *Allerg Immunol (Paris)* 1997;29:15–21.
- 24 Sabbah A, Sainte-Laudy J: Flow cytometry applied to the analysis of lymphocyte and basophil activation. *Allergy Clin Immunol Int* 1996;8:116–119.
- 25 Cozon JN, Ferrandiz J, Peyramond D, Brunet JL: Detection of activated basophils using flow cytometry for diagnosis in atopic patients. *Allergol Immunopathol* 1999;27: 162–167.
- 26 Sanz ML, Sanchez G, Gamboa PM, Vila L, Uasuf C, Chazot M, Dieguez I, De Weck AL: Allergen-induced basophil activation: CD63 cell expression detected by flow cytometry in patients allergic to *Dermatophagoides pteronyssinus* and *Lolium perenne*. *Clin Exp Allergy* 2001;30:1007–1013.
- 27 Paris-Kohler A, Demoly P, Persi L, Lebel B, Bousquet J, Arnoux B: In vitro diagnosis of cypress pollen allergy by using cytofluorimetric analysis of basophils (Basotest). *J Allergy Clin Immunol* 2000;105:339–345.
- 28 Saporta M, Kamei S, Persi L, Bousquet J, Arnoux B: Basophil activation during pollen season in patients monosensitized to grass pollens. *Allergy* 2001;56:442–445.
- 29 Hauswirth AW, Natter S, Ghannadan M, Majlesi Y, Scherthaner GH, Sperr WH, Bühring HJ, Valenta R, Valent P: Recombinant allergens promote the expression of CD203c on basophils in sensitized individuals. *J Allergy Clin Immunol* 2002;110:102–109.
- 30 Michova A, Abugalia M, Ivanova TS, Nikolov G, Taskov H, Petrunov B: Comparison of two flow cytometry methods for basophil degranulation in patients sensitized to grass pollen. *Allergy* 2006;61:1078–1093.
- 31 Setinova I, Haranova M, Honzova S: The basophil activation test in the diagnosis of birch pollen allergy. *Allergy Clin Immunol Int: J World Allergy Org* 2005;14(suppl 1): 527.
- 32 Pravtchanska-Semikova MP, Dimitrov MD, Baleva MP, Popov TA, Popova DN, Staevska M, Maleev V, Hristova S, Hristov G: Basophil activation test in the diagnosis of allergy to house dust mites and grass pollens – comparison with skin prick tests and specific IgE. Paris, EAACI Congress, 2003, p 370.
- 33 Gulbahar O, Mete N, Sin A, Kokuludag A, Sebik F: In vitro diagnosis of olive pollen allergy: the basophil activation test. *Allergy Clin Immunol Int: J World Allergy Org* 2005; 14(suppl 1):200.
- 34 Sabbah A, Plassais R, Grenapin S, Drouet M, Lauret MG, Loiry M, Sainte-Laudy J: Le test d'activation des basophiles par cytométrie en flux dans le diagnostic de l'allergie aux venins d'hyménoptères. *Allerg Immunol (Paris)* 1998;30:44–48.
- 35 Sainte-Laudy J, Sabbah A, Drouet M, Lauret MG, Loiry M: Diagnosis of venom allergy by flow cytometry. Correlation with clinical history, skin tests, specific IgE, histamine and leukotriene C4 release. *Clin Exp Allergy* 2000;30:1166–1171.
- 36 Freitag M, Höxtermann S, Freitag AP, Straube M, von Kobyletzki G, Altmeyer P, Seliska C: Flowzytometrische Messung der Aktivierung basophiler Granulozyten zur Diagnose der Wespengiftallergie. *Allergologie* 2001;24:2–9.
- 37 Platz I, Binder M, Marxer A, Lischka G, Valent P, Bühring HJ: Hymenoptera venom-induced upregulation of the basophil activation marker ecto-nucleotide pyrophosphatase/phosphodiesterase 3 in sensitized individuals. *Int Arch Allergy Immunol* 2001;126:335–342.
- 38 Lambert C, Guillox L, Dzviga C, Gourgaud-Massias C, Genin C: Flow cytometry versus histamine release analysis of in vitro basophil degranulation in allergy to Hymenoptera venom. *Cytometry B Clin Cytom* 2003;52:13–19.
- 39 Erdmann SM, Sachs B, Moll-Slodowy S, Merk HF: CD63-Expression der Basophilen in der Diagnostik der Wespengiftallergie und zur Verlaufskontrolle der spezifischen Immuntherapie. *Allergol J* 2003;12:S55–S67.
- 40 Erdmann SM, Sachs B, Kwiecién R, Moll-Slodowy S, Suer I, Merk HF: The basophil activation test in wasp venom allergy: sensitivity, specificity and monitoring specific immunotherapy. *Allergy* 2004;59:1102–1109.
- 41 Sturm GJ, Böhm E, Trummer M, Weiglhofer I, Heinemann A, Aberer W: The CD63 basophil activation test in Hymenoptera venom allergy: a prospective study. *Allergy* 2004;59: 1110–1117.
- 42 Eberlein-König B, Ring J: Diagnosis of IgE-mediated Hymenoptera venom anaphylaxis in patients with negative skin tests and negative RAST using cellular in vitro tests. *J Allergy Clin Immunol* 2004;111:1223–1224.

- 43 Eberlein-König B, Rakoski J, Behrendt H, Ring J: Use of CD63 expression as marker of in vitro basophil activation in identifying the culprit in insect venom allergy. *J Invest Allergol Clin Immunol* 2004;14:10–16.
- 44 Scherer K, Weber JM, Jermann TM, Krautheim A, Keefe D, de Weck AL, Bircher AJ: The use of cellular in vitro assays in the diagnosis of hymenoptera venom allergy. *J Allergy Clin Immunol* 2005;115:S108.
- 45 Scherer K, Weber JM, Jermann TM, Krautheim A, Tas E, Ueberschlag ME, Cammarata M, Bircher AJ: Cellular in vitro assays in the diagnosis of hymenoptera venom allergy. *Int Arch Allergy Immunol* 2008;146:122–132.
- 46 Eberlein-König B, Schmidt-Leidescher C, Rakoski J, Behrendt H, Ring J: In vitro basophil activation using CD63 expression in patients with bee and wasp venom allergy. *J Invest Allergol Clin Immunol* 2006;16:5–10.
- 47 Eberlein-König B, Varga R, Mempel M, Darso U, Behrendt H, Ring J: Comparison of basophil activation tests using CD63 or CD203c expression in patients with insect venom allergy. *Allergy* 2006;61:1084–1085.
- 48 Ebo DG, Hagendorens MM, Schuerwegh AJ, Beirens LM-N, Bridts CH, De Clerck LS, Stevens WJ: Flow-assisted quantification of in vitro activated basophils in the diagnosis of wasp venom allergy and follow-up of wasp venom immunotherapy. *Cytometry B Clin Cytom* 2007;72:196–203.
- 49 Stevens S, Drouet M, Lauret MG, Loiry M, Sabbah A: Basophil activation test using flow cytometry in Hymenoptera venom allergy. *Allerg Immunol (Paris)* 1999;31:11–14.
- 50 Kosnik M, Silar M, Bajrovic N, Music E, Korosec P: High sensitivity of basophils predicts side effects in venom immunotherapy. *Allergy* 2005;60:1401–1406.
- 51 Korosec P, Kosnik M: Predicting side-effects in venom immunotherapy by basophil activation: basophil sensitivity vs maximal response. *Allergy* 2007;62:81.
- 52 Eberlein-König B, Schmodt-Leidescher C, Begrendt H, Ring J: Predicting side-effects in venom immunotherapy by basophil activation? *Allergy* 2006;61:897.
- 53 Brown SG, Haas MA, Black JA, Parameswaran A, Woods GM, Hedde RJ: In vitro testing to diagnose venom allergy and monitor immunotherapy: a placebo-controlled, crossover trial. *Clin Exp Allergy* 2004;34:792–800.
- 54 Gober LM, Eckman JA, Sterba PM, Vasagar K, Schroeder JT, Golden DBK, Saini SS: Expression of activation markers on basophils in a controlled model of anaphylaxis. *J Allergy Clin Immunol* 2007;119:1181–1183.
- 55 Dankova E, Havranova M, Roztocilova J, Slavickova M, Koubova J, Honzova S: The basophil activation test: a great help in our outpatient clinic. Paris, EAACI Congress, 2003, p 228.
- 56 Dorn I, Eberhardt F, Hartwig D, Grabbe UJ: Diagnostik von Insektengiftallergien über die Messung von 203c. *Allergol J* 2005;14:45.
- 57 Ebo DG, Lechkar B, Schuerwegh AJ, Bridts CH, De Clerck LS, Stevens WJ: Validation of a two-color flow cytometric assay detecting in vitro basophil activation for the diagnosis of IgE-mediated natural rubber latex allergy. *Allergy* 2002;57:706–712.
- 58 Sanz ML, Gamboa PM, Garcia-Aviles C, Vila L, Dieguez I, Antepara I, de Weck AL: Flow-cytometric cellular allergen stimulation test in latex allergy. *Int Arch Allergy Immunol* 2003;130:33–39.
- 59 Erdmann SM, Moll-Slodowy S, Sauer I, Merk HF: CD63 expression on basophils as a tool for the diagnosis of latex allergy: sensitivity and specificity. Paris, EAACI Congress, 2003, p 371.
- 60 Boumiza R, Monneret G, Forissier MF, Savoye J, Gutowski MC, Powell WS, Bienvenu J: Marked improvement of the basophil activation test by detecting CD203c instead of CD63. *Clin Exp Allergy* 2003;33:259–265.
- 61 Hemery ML, Arnoux B, Dhivert-Donnadieu H, Rongier M, Barbotte E, Verdier R, Demoly P: Confirmation of the diagnosis of natural rubber latex allergy by the Basotest method. *Int Arch Allergy Immunol* 2005;136:53–57.
- 62 Sanz ML, Garcia-Aviles MC, Tabar AI, Anda M, Garcia BE, Barber D, Salcedo G, Rihs HP, Raulf-Heimsoth M: Basophil activation test and specific IgE measurements using a panel of recombinant natural rubber latex allergens to determine the latex allergen sensitization profile in children. *Pediatr Allergy Immunol* 2006;17:148–156.
- 63 Nettis E, Colanardi MC, Dambra PP, Capuzimati L, Loria MP, Ferrannini A, Vacca A, Tursi A: Flow cytometric basophil activation test: detection of CD63 expression as a useful aid to diagnosis of latex allergy. *Ann Allergy Asthma Immunol* 2006;97:715–716.
- 64 Moneret-Vautrin D, Sainte-Laudy J, Kanny G, Frémont S: Human basophil activation measured by CD63 expression and LTC4 release in IgE-mediated food allergy. *Ann Allergy Asthma Immunol* 1999;82:33–40.
- 65 Garcia-Aviles C, Sanchez-Lopez G, Sanz ML, Uasuf C, Dieguez I, Chazot M, de Weck AL: Flow cytometric allergen stimulation test (FAST) in the in vitro diagnosis of food allergy. *Allergy* 2000;55(suppl 63):128.
- 66 Diaz-Perales A, Sanz ML, Garcia-Casado G, Sanchez-Monge R, Garcia-Selles FJ, Lombarado M, Polo F, Gamboa PM, Barber D, Salcedo G: Recombinant Pru p 3 and natural Pru p 3, a major peach allergen, show equivalent immunologic reactivity: a new tool for the diagnosis of fruit allergy. *J Allergy Clin Immunol* 2002;111:628–633.
- 67 Erdmann SM, Heussen N, Moll-Slodowy S, Merk HF, Sachs B: CD63 expression on basophils as a tool for the diagnosis of pollen-associated food allergy: sensitivity and specificity. *Clin Exp Allergy* 2003;33:607–614.
- 68 Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS, Stevens WJ: Flow cytometric analysis of in vitro activated basophils, specific IgE and skin tests in the diagnosis of pollen-associated food allergy. *Cytometry B Clin Cytom* 2005;64:28–33.
- 69 Erdmann SM, Sachs B, Schmidt A, Merk HF, Scheiner O, Moll-Slodowy S, Sauer I, Kwiecien R, Maderegger B, Hoffmann-Sommergruber K: In vitro analysis of birch-pollen-associated food allergy by use of recombinant allergens in the basophil activation test. *Int Arch Allergy Immunol* 2005;136:230–238.
- 70 Caceres O, Gamboa P, Ahrazem O, Berber D, Garcia-Aviles G, Salcedo G, Sanz ML: Response to recombinant allergens in basophil activation test (BAT) and histamine release test (HRT) in patients with allergy to Rosaceae. *Allergy Clin Immunol: J World Allergy Org* 2005;14(suppl 1):366.
- 71 Gamboa PM, Caceres O, Antepara I, Sanchez-Monge R, Ahrazem O, Salcedo G, Berber D, Lombardero M, Sanz ML: Two different profiles of peach allergy in the north of Spain. *Allergy* 2007;62:408–414.
- 72 Ocmant A, Schandene L: Tests d'activation des basophiles dans les allergies à l'arachide et à l'oeuf. Paris, Meeting Tests D'Activation des Basophiles, Association Française de Cytométrie, 2006.
- 73 Ballmer BK, Weber JM, Vieths S, Wüthrich B: Predictive value of the sulfidoleukotriene release assay (CAST-ELISA) in oral allergy syndrome to celery, hazelnut and carrot. *J Invest Allergol Clin Immunol*, in press.
- 74 Gamboa PM, Sanchez-Monge R, Diaz-Perales A, Salcedo G, Ansotegui J, Sanz ML: Latex-vegetable syndrome due to custard apple and aubergine: new variations of the hevein syndrome. *J Invest Allergol Clin Immunol* 2005;15:308–311.
- 75 Gonzales-Munoz M, Luque R, Nauwelaers F, Monco I: Detection of *Anisakis simplex*-induced basophil activation by flow cytometry. *Cytometry B Clin Cytom* 2005;68:31–36.
- 76 Schäd SG, Trcka J, Vieths S, Scheurer S, Conti A, Brocker EB, Trautmann A: Wine anaphylaxis in a German patient: IgE-mediated allergy against a lipid transfer protein from grapes. *Int Arch Allergy Immunol* 2005;136:159–164.
- 77 Vassilopoulou E, Bossios A, Papadopoulos NG, Volonski E, Mannoussakis E, Gourgiotis D, Papageorgiou PS: Flow cytometric basophil allergen stimulation test (FAST) in food allergy. Paris, EAACI Congress, 2003, p 187.

- 78 Stevens WJ, Ebo DG, De Clerck LS, Bridts CH: Anaphylaxis to sesame (*Sesamum indicus*) seed and sesame oil. *J Allergy Clin Immunol* 2002;109:250.
- 79 Shreffler WG: Evaluation of basophil activation in food allergy: present and future applications. *Curr Opin Allergy Clin Immunol* 2006;6:226–233.
- 80 Ebo DG, Ahrazem O, Lopez-Torres G, Bridts CH, Salcedo G, Stevens WJ: Anaphylaxis from mandarin (*Citrus reticulata*): identification of potential responsible allergens. *Int Arch Allergy Immunol* 2007;144:39–43.
- 81 Leynadier F, Rajoely B, Ghazouani E, Pecquet C, Abuaf N: The diagnosis of anaphylactic reactions to muscle relaxants by flow cytometry. *J Allergy Clin Immunol* 1996;97:344.
- 82 Abuaf N, Rajoely B, Ghazouani E, Levy DA, Pecquet C, Chabane H, Leynadier F: 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.
- 83 Monneret G, Benoit Y, Debar AL, Gutowski MC, Topenot I, Bienvenu J: Monitoring of basophil activation using CD63 and CCR3 in allergy to muscle relaxant drugs. *Clin Immunol* 2002;102:192–199.
- 84 Sudheer PS, Hall JE, Read GF, Rowbottom AW, Williams PE: Flow cytometric investigation of peri-anaesthetic anaphylaxis using CD63 and CD203c. *Anaesthesia* 2005;60:251–256.
- 85 Kvedariene V, Kamey S, Ryckwaert Y, Rongier M, Bousquet J, Demoly P, Arnoux B: Diagnosis of neuromuscular blocking agent hypersensitivity reactions using cytofluorimetric analysis of basophils. *Allergy* 2006;61:311–315.
- 86 Ebo DG, Bridts CH, Hagendorens MM, Mertens CH, De Clerck LS, Stevens WJ: Flow-assisted diagnostic management of anaphylaxis from rocuronium bromide. *Allergy* 2006;61:935–939.
- 87 Nettis E, Colanardi MC, Dambra PP, Capuz-zimati L, Loria MP, Ferrannini A, Vacca A, Tursi A: Flow cytometric basophil activation of CD63 expression as a useful aid to diagnosis of latex allergy. *Ann Allergy Asthma Immunol* 2006;97:715–716.
- 88 Sanz ML, Gamboa PM, Antepara I, Uasuf C, Vila L, Garcia-Aviles C, Chazot M, de Weck AL: Flow-cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics. *Clin Exp Allergy* 2002;32:277–286.
- 89 De Weck AL, Gamboa PM, Sanz ML: Clinical evaluation of in vitro tests in diagnosis of immediate allergic reactions to betalactam antibiotics. *Allergy Clin Immunol Int* 2002;14:185–192.
- 90 Torres MJ, Padial A, Mayorga C, Fernandez T, Sanchez-Sabate E, Cornejo-Garcia A, Antunez C, Blanca M: The diagnostic interpretation of basophil activation test in immediate allergic reactions to betalactams. *Clin Exp Allergy* 2004;34:1768–1775.
- 91 Arnoux B, Kvedariene V, Rongier M, Bousquet J, Demoly P: β -Lactam allergy in clinical practice. Relevance of clinical history and/or laboratory test. *J Allergy Clin Immunol* 2004;113:s71.
- 92 Gamboa PM, Garcia-Aviles MC, Urrutia I, Antepara I, Esparza R, Sanz ML: Basophil activation and sulfidoleukotriene production in patients with immediate allergy to betalactam antibiotics and negative skin tests. *J Invest Allergol Clin Immunol* 2004;14:278–283.
- 93 Kvedariene V, Arnoux B, Rongier M, Bousquet PF, Demoly P: Benefit of the basophil activation test for the diagnosis of immediate drug hypersensitivity reaction. *Allergy Clin Immunol Int: J World Allergy Org* 2005;14(suppl 1):128.
- 94 Erdmann SM, Ventocilla S, Moll-Slodowy S, Sauer L, Merk HF: Basophil activation tests in the diagnosis of drug reactions. *Hautarzt* 2005;56:38–43.
- 95 De Weck AL, Sanz ML, Gamboa PM, Aberer W, Bilo B, Blanca M, Torres M, Mayorga L, Campi P, Drouet M, Sainte-Laudy J, Romano A, Jermann T, Weber JM, ENDA Group: Diagnosis of immediate-type betalactam allergy in vitro by flow cytometry (Flow-CAST) and sulfidoleukotriene production. A multicentric study. Vienna, EAACI Congress, 2006, p 686.
- 96 Rodriguez-Trabado A, Camara Higon C, Porcel Carreno SL, Rodriguez Martin E, Fletes Peral C, Pereira Narro G, Jimenez Timon T, Hernandez Abeija FJ, Fernandez Pereira L: Anaphylaxis caused by cloxacillin: diagnosis with seriated analysis by way of basophil activation test. *Allergy Asthma Proc* 2006;27:269–272.
- 97 Blanca M, Mayorga C, Torres MJ, Reche M, Moya MC, Rodriguez JL, Romano A, Juarez C: Clinical evaluation of Pharmacia CAP system RAST FEIA amoxicilloyl and benzylpenicilloyl in patients with penicillin allergy. *Allergy* 2001;56:862–870.
- 98 Fontaine C, Mayorga C, Bousquet PJ, Arnoux B, Torres MJ, Blanca M, Demoly P: Relevance of the determination of serum-specific IgE antibodies in the diagnosis of immediate β -lactam allergy. *Allergy* 2007;62:47–52.
- 99 Gamboa PM, Sanz ML, Caballero MR, Antepara I, Urrutia I, Jauregui I, Gonzales G, Dieguez I, de Weck AL: Use of CD63 expression as a marker of in vitro basophil activation and leukotriene determination in met-amizol allergic patients. *Allergy* 2003;58:312–317.
- 100 Sanz ML, Gamboa P, de Weck AL: A new combined test with flowcytometric basophil activation and determination of sulfidoleukotrienes is useful for in vitro diagnosis of hypersensitivity to aspirin and other nonsteroidal anti-inflammatory drugs. *Int Arch Allergy* 2005;136:58–72.
- 101 Gamboa P, Sanz ML, Caballero MR, Urrutia I, Antepara I, Esparza I, de Weck AL: The flow-cytometric determination of basophil activation induced by aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) is useful for in vitro diagnosis of the NSAID hypersensitivity syndrome. *Clin Exp Allergy* 2004;34:1448–1457.
- 102 De Weck AL, Sanz ML, Gamboa PM, Aberer W, Sturm G, Blanca M, Torres M, Mayorga L, Correia S, Kowalski M, Medralla W, Szczeklik A, ENDA Group: Diagnosis of hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs) in vitro by flowcytometry (Flow-CAST) and sulfidoleukotriene assay. A multicentric study. Vienna, EAACI Congress, 2006, p 654.
- 103 De Weck AL, Sanz ML, Gamboa PM: Further considerations on the mechanism of NSAID hypersensitivity; in Ring J, Holgate S, Kay B (eds): CIA Symposium. Göttingen, Hogrefe & Huber, in press.
- 104 De Weck AL, Sanz ML, Gamboa P: New pathophysiological concepts on aspirin hypersensitivity (Widal syndrome): diagnostic and therapeutic consequences (in French). *Bull Acad Natl Med* 2005;189:201–218.
- 105 De Weck AL, Gamboa PM, Esparza B, Sanz ML: Hypersensitivity to aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs). *Curr Pharm Des* 2006;12:3347–3458.
- 106 Sainte-Laudy J: Nine cases of suspected IgE-mediated anaphylaxis induced by aspirin. *Allergy Clin Immunol Int* 2002;14:220–222.
- 107 Malbran A, Yeyati E, Rey GL, Galassi N: Diclofenac induces basophil degranulation without increasing CD63 expression in sensitive patients. *Clin Exp Immunol* 2007;147:99–105.
- 108 De Weck AL, Sanz ML: Cellular allergen stimulation test (CAST) 2003, a review. *J Invest Allergy Clin Immunol* 2004;14:253–273.
- 109 Le Pabic F, Sainte-Laudy J, Blanchard N, Moneret-Vautrin DA: First case of anaphylaxis to iodinated povidone. *Allergy* 2003;58:826–827.
- 110 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.

- 111 Ebo DG, Haine SE, Hagendorens MM, Bridts CH, Conraads VM, Vorlat A, Vrints CJ, De Clerck LS, Stevens WJ: Hypersensitivity to nadroparin calcium: case report and review of the literature. *Clin Drug Investig* 2004;24:421–426.
- 112 Caballero MR, Fernandez-Benitez M: Allergy to heparin: a new in vitro diagnostic technique. *Allergol Immunopathol (Madrid)* 2003;31:324–328.
- 113 Ebo DG, Bridts CH, Stevens WJ: Anaphylaxis to an urethral lubricant: chlorhexidin as the 'hidden' allergen. *Acta Clin Belg* 2004;59:358–360.
- 114 Ebo DG, Bridts CH, Stevens WJ: IgE-mediated anaphylaxis from chlorhexidine: diagnostic possibilities. *Contact Dermatitis* 2006;55:301–302.
- 115 Ebo DG, Schuerwegh A, Stevens WJ: Anaphylaxis to starch. *Allergy* 2000;55:1098–1099.
- 116 Ebo DG, Wets RD, Spiessens TK, Bridts CH, Stevens WJ: Flow-assisted diagnosis of anaphylaxis to patent blue. *Allergy* 2005;60:705–706.
- 117 Scherer K, Studer W, Figueiredo V, Bircher AJ: Anaphylaxis to isosulfan blue and cross-reactivity to patent blue V: case report and review of the nomenclature of vital blue dyes. *Ann Allergy Asthma Immunol* 2006;96:497–500.
- 118 Gamboa PM, Sanz ML, Urrutia I, Jauregui I, Antepara I, Dieguez I, De Weck AL: CD63 expression by flow cytometry in the in vitro diagnosis of allergy to omeprazole. *Allergy* 2003;58:538–539.
- 119 Ebo DG, Goossens S, Opsomer F, Bridts CH, Stevens WJ: Flow-assisted diagnosis of anaphylaxis to hyaluronidase. *Allergy* 2005;60:1333–1334.
- 120 Bauer C, Opple T, Rueff F, Przybilla B: Anaphylaxis to viscotoxins of mistletoe (*Viscum album*) extracts. *Ann Allergy Asthma Immunol* 2005;94:86–89.
- 121 Apostolou E, Deckert K, Puy R, Sandrini A, de Leon MP, Douglass JA, Rolland JM, O'hehir RE: Anaphylaxis to Gelofusine confirmed by in vitro basophil activation test: a case series. *Anaesthesia* 2006;61:264–268.
- 122 Shields MD, Mirakhur RK, Crockard AD, Edfar GD: The use of basophil activation to diagnose allergy to Gelofusine. *Anesthesia* 2006;61:716–717.
- 123 Caceres Calle O, Fernandez-Benitez M: Allergy to dexchlorpheniramine. Study of a case. *Allergol Immunopathol (Madr)* 2004;32:306–309.
- 124 Orta M, Ordoqui E, Aranzabal A, Fernandez C, Bartolome B, Sanz ML: Anaphylactic reaction after artificial insemination. *Ann Allergy Asthma Immunol* 2003;90:446–451.
- 125 Nopp A, Johansson SG, Ankerst J, Bylin G, Cardell LO, Gronneberg R, Irander K, Palmqvist M, Oman H: Basophil allergen threshold sensitivity, a useful approach to anti-IgE treatment efficacy evaluation. *Allergy* 2006;61:298–302.
- 126 Ebo DG, Lechkar B, Schuerwegh AJ, Bridts CH, De Clerck LS, Stevens WJ: Validation of a two-color flow cytometric assay detecting in vitro basophil activation for the diagnosis of IgE-mediated natural rubber latex allergy. *Allergy* 2002;57:706–712.
- 127 Sanz ML, Gamboa PM, Garcia-Aviles C, Vila L, Dieguez I, Antepara I, de Weck AL: Flow-cytometric cellular allergen stimulation test in latex allergy. *Int Arch Allergy Immunol* 2003;130:33–39.
- 128 Mari A: IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity. *Int Arch Allergy Immunol* 2002;129:286–295.
- 129 De Swertdt A, Van Den Keybus C, Kasran A, Cadot P, Neyens K, Coorevits L, Kochuyt AM, Degreef H, Ceuppens JL: Detection of basophil-activating IgG autoantibodies in chronic idiopathic urticaria by induction of CD63. *J Allergy Clin Immunol* 2005;116:662–667.
- 130 Frezzolini A, Provini A, Teofoli P, Pomponi D, De Pita O: Serum-induced basophil CD63 expression by means of a tricolour flow cytometric method for the in vitro diagnosis of chronic urticaria. *Allergy* 2006;61:1071–1077.
- 131 Grattan CEH, Walpole D, Francis DM, Nimi DM, Dooston G, Edler S, Corbett MF, Barr RM: Flow cytometric analysis of basophil numbers in chronic urticaria: basopenia is related to serum histamine releasing activity. *Clin Exp Allergy* 1997;27:1417–1424.
- 132 Gyimesi E, Sipka S, Danko K, Kiss E, Hidvegi B, Gal M, Hunyadi J, Irinyi B, Szegedi A: Basophil CD63 expression assay on highly sensitized atopic donor leukocytes – a useful method in chronic autoimmune urticaria. *Br J Dermatol* 2004;151:388–396.
- 133 Szegedi A, Irinyi B, Gal M, Hunyadi J, Danko K, Kiss E, Sipka S, Szegedi G, Gyimesi E: Significant correlation between the CD63 assay and the histamine release assay in chronic urticaria. *Br J Dermatol* 2006;155:67–75.
- 134 Vasagar K, Vonakis BM, Gober LM, Viksman A, Gibbons SP Jr, Saini SS: Evidence of in vivo basophil activation in chronic urticaria. *Clin Exp Allergy* 2006;36:770–776.
- 135 Vermeulen C, Mathelier-Fusade P, Bayrou O, Abuaf N, Pecquet C, Levy D, Leynadier F: Response of basophils to autologous serum from patients with chronic urticaria. *J Allergy Clin Immunol* 2002;109:S88.
- 136 Wedi B, Novacovic V, Koerner M, Kapp A: Chronic urticaria serum induces histamine release, leukotriene production and basophil CD63 surface expression – inhibitory effects of anti-inflammatory drugs. *J Allergy Clin Immunol* 2000;105:552–560.
- 137 Yasnovsky KM, Dreskin SC, Efav B, Schoen D, Vedanthan PK, Alam R, Harbeck RJ: Chronic urticaria sera increase basophil CD203c expression. *J Allergy Clin Immunol* 2006;117:1430–1434.
- 138 Zuberbier T, Henz BM, Fiebiger E, Maurer D, Stingl G: Anti-FcεR1α serum autoantibodies in different subtypes of urticaria. *Allergy* 2000;55:951–954.
- 139 Renz H, Becker WM, Bufe A, Kleine-Tebbe J, Raulf-Heimsoth M, Saloga J, Werfel T, Worm M: In-vitro-Allergiediagnostik. Positionspapier der Deutschen Gesellschaft für Allergologie und klinische Immunologie. *Allergo J* 2002;11:492–506.
- 140 Hamilton RG, Adkinson NF: In vitro assays for the diagnosis of IgE-mediated disorders. *J Allergy Clin Immunol* 2004;114:213–225.
- 141 First International Allergy Flow Cytometry Workshop. Pamplona, October 19–20, 2007.
- 142 Plebani M, Borghesan F, Basso D, Faggian D: Receiver-operating characteristic (ROC) curves: a fundamental tool for improving the clinical usefulness of in vitro IgE tests. *Allergy* 1996;51:407–411.
- 143 Kalayci O, Saraclar Y, Adalioglu G, Sekerel B, Tuncer A: The effect of cetirizine on sulfidoleukotriene production by blood leukocytes in children with allergic rhinitis. *Allergy* 1995;50:964–969.