Review Article

Cellular Allergen Stimulation Test (CAST) 2003, a review

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Summary. Specific diagnosis of immediate type allergies, such as rhinoconjunctivis, asthma, urticaria/angioedema and anaphylaxis, particularly when IgE-mediated, traditionally rests on prick and/or intradermal skin tests and, since about 30 years, on the determination of allergen specific IgEs.
Some cellular tests, i.e. tests determining the reactivity of blood cells in vitro, particularly basophils, to allergens, have been available for many years. The determination of histamine release has been widely used in allergy pathophysiological research but its routine application in allergy diagnosis has been restricted to few groups. Basophil degranulation, as determined by microscopic examination, was promoted by some groups in the 1980's but has been largely abandoned since around 10 years ago; an alternative cellular test, based on the determination of sulfidoleukotrienes (LTC4, LTD4, LTE4) produced by IL-3 primed basophils stimulated by allergens in vitro, has been proposed. This test became available commercially in 1993 under the name of CAST (Bühlmann Laboratories, Allschwil, Switzerland).
The CAST assay has been used in allergy diagnosis in a variety of indications, such as inhalation allergies, allergies to insect venoms, foods, occupational allergens and various drugs. A large number of reports on CAST diagnostic value, however, have been anecdotal. A meta-analysis of validated and well controlled studies encompasses 37 studies, 1614 patients and 1145 controls. This should definitely establish the value of this diagnostic test, particularly in instances where other in vitro or in vivo diagnostic tests are not reliable, such as food or drug allergies, as well as in non-IgE- mediated immediate hypersensitivity reactions.
However, a number of questions about the CAST diagnostic assay are still open or have not been systematically explored. This may explain, in addition to the practical limitations inherent to all allergy cellular tests, why CAST has not yet become a very widely used assay worldwide, having gained broad acceptance in some countries but not in others.

Key words: Sulphidoleukotriens, CAST, allergy, in vitro diagnosis

Some cellular tests, i.e. tests determining the reactivity of blood cells in vitro, particularly basophils, to allergens, have also been available for many years. The determination of histamine release has been widely used in allergy pathophysiological research but its routine application in allergy diagnosis has been restricted to few groups [1,2]. Basophil degranulation, as determined by microscopic examination, was promoted by some groups in the 1980's [3,4] but has been largely abandoned since.

Around 10 years ago, an alternative cellular test, based on the determination of sulfidoleukotrienes (LTC4, LTD4, LTE4) produced by IL-3 primed basophils stimulated by allergens in vitro, has been proposed by one of us [5-8]. This test became available commercially in 1993 under the name of CAST (Bühlmann Laboratories, Allschwil, Switzerland). While the majority of studies in the literature have been performed with this test, some have used other commercially available anti-sulfidoleukotrienes (sLT)

antibodies, usually of more restricted specificity (e.g. anti-LTC4, Cayman Laboratories).

Despite the fact that, particularly in Europe, several groups have published over the years positive and clinically validated studies on the use of CAST in allergy diagnosis for various indications, particularly for insect venoms and drugs, other reports have been definitely negative [9]. This apparent controversy has been reinforced by some incomplete overviews [10,11] and recently by a position paper from a study group of the German Society for Allergology and Clinical Immunology, which basically concludes that for most indications, there are not enough studies to recommend the CAST assay for routine allergy diagnosis [12]. Indeed, only three studies on CAST results are quoted in that position paper, one of them negative [9,13,14] although at that time more than 20 thoroughly controlled and clinically validated studies had been published. It may be difficult for many to objectively assess the literature, since only one review in German from 1997 has up to now been published [15]. In addition, many of the earlier publications were anecdotal, reporting relatively few cases. Many reports have appeared in other languages than English and/or are not available as Medline quotations. We have therefore found it useful to attempt a better informed assessment, and to present here the first comprehensive review of published CAST results in various clinical diagnostic indications.

This review includes, on the one hand and for the clinical indications of inhalants, food, venoms, occupational and drugs allergies, only 35 publications (with a total 504 patients investigated) which we consider "anecdotal", i.e. based on relatively few cases (less than 10) and/or insufficiently controlled. On the other hand, for the same diagnostic indications we have reviewed 37 studies, with a total 1614 patients, which we consider clinically validated, the allergy diagnosis having been firmly established by other diagnostic criteria (history, skin tests, specific IgE determinations and, when indicated, provocation tests). Such studies have also included suitable control groups with a sufficient number of individuals (10 or more), totalling 1145 controls.

Recently, another cellular allergy diagnostic test, the flowcytometric basophil activation test (BAT or FAST)[1] has been developed and used in a number of clinical diagnostic indications similar to those of CAST. These results have been recently reviewed [16,17]. Since the combination of flow cytometric basophil activation and sLT determinations yields in some indications complementary and improved diagnostic results, these will also be briefly discussed below[2] .

Allergy to inhalant allergens

In the early studies about CAST, the focus was set on classical aeroallergens such as grass pollens and house dust mites, in order to assess the usefulness and reliability of the test in IgE-mediated allergy and its position relative to other diagnostic tests, such as skin tests, determination of allergen-specific IgEs and histamine release test. After the first anecdotal studies, four validated studies encompassing 386 patients and 247 controls, particularly with grass pollen (Lolium perenne, timothy) and with house dust mites (Dermatophagoides pteronyssinus) have confirmed the high sensitivity and specificity of CAST in these indications (Table 1). In particular, the CAST test appears to be more sensitive than histamine release [18,19]. Since in most instances the patient's history, skin tests and determination of allergen-specific IgE suffice to establish the diagnosis and to provide indication for immunotherapy, the CAST, as an additional and expensive diagnostic test, is rarely justified and has accordingly not found its way into routine allergology practice.

The matter might be different, however, in allergy diagnosis of early infancy, where skin tests are more difficult to perform and where determinations of allergen-specific IgE are less reliable and correlate less closely with the clinics. CAST has been tested repeatedly in infant and children populations [20,21] but only one study has compared its diagnostic efficiency in comparison to skin tests and to allergen-specific IgE determinations and has concluded that CAST was not more sensitive for allergy diagnosis in infants [22]. Nevertheless, in individual cases CAST has shown that it may be positive to inhalation and food allergens in infants developing atopy while skin tests and specific IgE determinations are still negative [15]. In view of the increasing prevalence of paediatric IgE-mediated allergies and of the importance of early detection for secondary prevention, additional studies would be most desirable.

Detection of atopy

Following the same line of thought, early detection of atopy and of the genetically conditioned ability to develop high IgE levels in response to environmental or food allergens has been increasingly emphasized. With the exception of the determination of egg or milk-specific IgE at the age of 6 – 12 months [23], other parameters such as the determination of total or specific IgE or of allergen-specific T lymphocytes reactivity in cord blood have proven disappointing and of poor predictive value for the development of atopy.

In this respect, one study, although interpreted by its authors in a rather negative way [20] points out to

[1] Commercially available under the name of FLOW-CAST (Bühlmann Laboratories, Allschwil, Switzerland) or BASOTEST (Beckton-Dickinson).

[2] Commercially available combined FAST and CAST under the name of CAST Combi (Bühlmann Laboratories, Allschwil, Switzerland).

Table 1. Inhalant allergens

Ref No	Author	Allergens	Patients	Nb	Result	Controls	Nb	Results	Positivity criteria	Comments
A. Anecdotal studies										
6	De Weck et al., 1992	5 inhalant allergens	Rhinitis/Asthma	5	5 pos. . corr with ST 1.0					
5	De Weck et al,1992	5 inhalant allergens	Rhiniti/asthma	24	corr ST 0.76; corr sIgE: 0.66					
7	De Weck et al, 1993	5 inhalant allergens	Rhinitis/ Asthma	38	corr St 0.85, corr sIgE 0.72					
			HDM allergics	18	corr sLT to HDM/Anti-IgE 0.86					
117	Cosachov et al.,1995	GP, Ragweed, Moulds	Allergics	6	corr ST; corr sIgE good mean sLT 561 pg/ml					
113	Hipler, 1994	GP	Allergics	10	high corr sLT / ST					intra, interassay CV < 10%
102	Sainte-Laudy, 1995	inhalant allergens (Gp5,cat,HDM)	Allergics	7	5 SIgE/sLT pos.(520-610 pg) 2 sIgE neg/sLT pos				sLT 100 pg/ml >Bckg	SE sLT > HR
103	Siridopoulos,1995	HDM,GP	allergics	11	low SE	Healthy	4	high SP	200 pg > Bckg	
18,19	Ferrer et al., 1996	GP, HDM	HDM allergics	23	significant sLT in patients					
			GP allergics	10	corr sLT/HR 0.44					
132	Sainte-Laudy, 1996	HDM	HDM	5	5/5 pos (sLT 130-710 pg/ml)	Non sensitized human basophils		high SP		human baso sensitized by passive serum transfer (heat labile) inhibition HR but not sLT by high dose Ag
134	Medrala et al, 1997	GP	Allergics	23	20/23 (SE 87%) -mean:1671 pg/ml				sLT 200 pg/ml > Bckg	
B. Validated studies										
111	Medrala et al, 1997	HDM	Allergics	23	21/23 (SE 91%)-mean:1521 pg/ml	Healthy	15	12 sLT,ST neg (SP 100%) 3 sLT,ST pos (< 300 pg/ml)	sLT 200 pg/ml > Bckg	
98	Ferrer et al, 1996	HDM	Allergics (ST pos)	62	sLT pos.-mean 1731 pg/ml	Atopics (HDM ST neg)	30	mean SLT 291 pg/ml		corr HR 0.83; corr sIgE 0.4
					coor ST: 0.42; corr sIgE 0.40 high SE	Healthy	12	mean sLT 55 pg/ml		corr ST 0.49 no corr with asthma severity
18	Ferrer et al, 1996	GP	Allergics (ST pos)	54	corr ST 0.50; mean sLT 2123 pg/ml	Atopics (GP ST neg)	38	mean sLT 940 pg/ml		corr HR 0.67, corr sIgE/ST 0.5 higher sLT in pollen season
						Healthy	9	mean sLT 62 pg/ml		sLTT < by age > 40 yrs
135	Rossi et al., 1998	HDM Dpt, Df	Rhiniis/Asthma (ST pos)	247	Dpt SE 71%,mean sLT 969/pg/ml Df SE 64%-mean SLT 1123 pg/ml	Healthy (ST, sIgE neg)	137	Dpt SP 87 %,mean SLT 240 pg/ml Df SP 71 %, mean sLT 249 pg/ml	defined by ROC curve Dpt: 470 pg Df: 523 pg	

the potential use of CAST as a screening test in cord blood. Among 13 children with family history of atopy, all were positive in CAST to a mixture of classical paediatric allergens (TOP CAST mixture of 21 allergens), while 24 out of 91 children with negative family history were also CAST positive. The authors concluded to poor specificity, but in view of the fact that about 50% of the infants ultimately developing atopy have no direct family history and that the children in that study were not followed up, a negative conclusion is not yet justified. This study shows that evidence of sensitisation is present already at the time of birth and basophils may release mediators in an allergen-specific way. Further CAST studies of newborns, if possible combined with flowcytometric determination of basophil activation and clinical follow up would be very desirable.

Another potential use of CAST for the detection of atopy has been as a screening test in children and adults, mostly using a mixture of allergens (TOP-CAST).

Among several such studies (Table 2), three validated ones encompassing 192 patients [24,25,26] have shown that CAST is indeed very reliable as a screening test for IgE-related atopy, to the same or even higher degree than screening for multiple allergen-specific IgEs (e.g. Phadiatop) [24,25,27] but whether this holds in infants has been questioned [22]. CAST could theoretically be used for mass blood screening, with greater reliability and logistical simplification than skin tests [28], particularly if it were automated.

Food allergy

Although IgE-mediated food allergies represent an important proportion of allergic diseases, their diagnosis is markedly more difficult than that of allergies to aeroallergens. Food allergies may take several forms, from immediate-type symptoms, such as anaphylaxis, urticaria, angioedema, asthma or rhinitis, to more delayed manifestations often including gastro-intestinal symptoms. Beyond history elaboration, which may be imprecise and difficult, skin tests and determination of specific IgEs are often considered relatively unreliable [29]. For some foods (e.g. celery, hazelnut, carrot), the sharing of epitopes with some pollens (e.g. birch) often make the determination of specific IgEs meaningless, since immunological cross-reactivity is not associated with clinical cross-reactivity [30].

Table 2. Detection of atopy

Ref No	Author	Allergens	Patients	Nb	Result	Controls	Nb	Results	Positivity criteria	Comments
24,27	Derer, 1995	TC mix	HDM/GP allergics	52	SE 86 %			SP 91 %		corr Phadiatop 0.88
119,14	Shichijo,1995	HDM,a-IgE	HDM Atopics	13	sLT > to HDM, a-IgE	Healthy	5	IL3 does not increase sLT from normal donors		sLT > in patients with HR 50% corr HR +IL3 0.83; -IL3 o.58
121	Zuberbier, 1996	a-IgE	chronic urticaria	8	mean sLT 586 pg/ml	Healthy	9	mean sLT 594 pg/ml		HR < in patients IL-3 (20 ng/ml > sLT to a-IgE
25	Medrala,1997	TC mix	HDM/GP allergics	24	24/24 (SE 100 %) at Ag 100 ng/ml	Healthy	16	4 sLT, ST pos, 11 SLT, ST neg 1 sLT neg, ST pos	sLT 200 pg/ml > Bckg	sLT, ST pos controls < 1000 pg/ allergics < 2000 pg/ml
26	Ferrer, Sanz 1998	HDM Dpt GP Lolp	Rhinitis/asthma Rhinitis/asthma	62 54	sLT to a-IgE ~2000 pg/ml corr sLTa-IgE/Ag 0.8	Healthy	12	sLT a-IgE ~700 pg/ml		14.5 % non responders to a-IgE IL-3 enhances sLT > HR
20	Szepfalusi , 1999	TC mix	Allergic children Cord blood, family pos Cord blood, family neg	56 13 91	45/56 pos to a-IgE Mean sLT to TC: 1049 pg/ml 13 sLT pos: to TC: 419 pg/ml 24 sLT pos.				sLT 200 pg/ml > Bckg	 sLT to a-IgE corr to IgE level; sLT to TC does not
22	Tridon , 1999	TC mix ?	Asthmatics 1-3 yrs old (17 without atopy yet)	44	sLT SE: 22%(6/27) sIgE SE 52%: ST SE 29%			high SP	sLT 200 pg/ml > Bckg	sLT pos only when ST or sIgE pos

Table 3. Food allergens

Ref No	Author	Allergens	Patients	Nb	Result	Controls	Nb	Results	Positivity criteria	Comments
A. Anecdotal studies										
113	Hipler,1995	egg,lobster	allergic	4	4 sLT,ST,sIgE pos	Healthy	1	sLT, ST, sIgE neg		
137	Steinmann, 1997	egg	AD children	17	10 Prov pows, sLT,ST,sIgE pos 4 Prov pos , sLT,ST,sIgE neg 3 Prov neg, sLT,ST, sIgE neg				pos DBPCFC	
106	Vila et al, 1998	chicken meat	OAS	1	sLT, sIgE,HR,ST pos					
34	Diaz-Perales, 2003	Peach,rPru p3 (ST,sIgE pos)	Peach allergics	10	9 /10 pos (SE 90 %)	Healthy	5	sLT SP 100%		SLT > HR (SE 50 %) rec Pru p3 = natural Pru p3
B. Validated studies										
36	Moneret-Vautrin ,1999	various foods	urticria,asthma anaphylaxis	27	22/34 sLT pos (SE 85 %) 18/31 BAT pos, sIgE SE 91%	Healthy	24	1/35 sLT pos (SP 100 %) 1/34 BAT pos(SP 100%)	sLT 100 pg/ml > Bckg Bckg < 100 pg/ml	sLT OK 24 hrs after sampling high spontaneous HR (7/11) passive serum transfer when sIgE < 35 kU > 3.5 kU
33	Vila et al, 1999	various foods	urticaria 9 anaphylaxis 15 rhinitis 5 OAS 13 exercise 5 gastrointest 3	40	sLT SE 81%,sIgE SE 77 % HR SE 84.5 %, 33 Prov pos mean sLT a-IgE 1327 pg/ml mean sLT Ag 836 pg/ml	Healthy Atopics no food allergy	20 15	sLT (SP 98 %) mean sLT a-IgE 1059 pg/ml mean sLT Ag 95 pg/ml sIgE (SP 95%) mean sLT a-IgE 1630 pg/ml mean sLT Ag 231 pg/ml	cutoff from ROC curves sLT 110 pg/ml non atopics sLT 250 pg/ml atopics	sLT pos in anaphylaxis corr ST 0.50; corr sIgE 0.35 corr HR 0.67 7 atopic patients pos sLT,ST HR, sIgE but neg DBPCFC Prov !
30	Ballmer,2003	hazelnut, celery,carrot	OAS Prov pos Birch allergics sT.sIgE pos Prov neg	43 41	sLT SE 70%(10ug Ag) sLT SE 84% (100 ug Ag) sLT SE 12 % (10ug Ag) sLT SE 32% (100ug Ag)	Healthy Birch allergices Prov neg	20 42	sLT SP 100% sLT SP 88 % (10ug Ag) sLT SP 68% (100 ug Ag)		sIgE SE 66%

Table 4. Insect Venoms.

Ref N	Author	Allergens	Patients	Nb	Result	Controls	Nb	Results	Positivity criteria	Comments
A. Aneccdotal studies										
108	Maly, 1994	bee,wasp	bee/wasp allergics	15	corr ST 0.6; corr sIgE 0.46 corr HR 0.36 - SE 87 %	Healthy	10	SP 94 %	> mean controls + 2 SD	sLT and HR dissociated
103	Siridopoulos,1995	bee,wasp	chidren	5	5/5 sLT pos	Healthy	6	several positive controls	nr	
113	Hippler, 1995	bee,wasp	bee/wasp allergics	14	almost complete corr with history, sIgE				nr	
107	Sabbah , 1997	mosquito	anaphylaxis	1	sLT (637 pg) , BAT , SIgE pos			sLT 24 pg/ml		
109	Pereira-Santos , 2002	bee,wasp	bee allergics	14	sLT SE:93%; ST SE 71% sIgE SE 71%	atopic	5	> 200 pg/ml		CAST pos in ST, sIgE neg
40,4	Eberlein-König , 2003	bee.wasp	bee/wasp allergics	14	wasp sLT SE 86 % bee sLT SE 50 %	Healthy sIgE neg	5	all sLT, sIgE neg	sLT > 681 pg/ml (bee) sLT > 864 pg/ml (wasp)	6/28 sT neg, BAT and CAST pos sLT corr BAT 0.79
B. Controlled studies										
112	Höxtermann,1995	wasp	wasp allergics	25	sLT SE 92 % corr ST 0.88;corr sIgE 0.84 sLT bee SE 100%; wasp SE 83%	Healthy sIgE neg	10	sLT SE 90%	> mean controls + 2 SD	corr sLT/sIgE 0.94 corr sLT/ST 0.88
37,1	Maly , 1996,1997	bee, wasp	bee/wasp allergics	23	17 ST pos SLT 88 % HR 59% 6 ST neg , SLT 66% corr ST 0.6; corr sIgE o.46	Healthy	10	sLT SP 90 % bee SP 77%; wasp SP 100%	> mean controls + 2 SD	dissociation sLT and HR corr ST : sLT > HR
13	Cahen , 1997	bee 31 wasp 27 ?? 8	bee/wasp allergics ST pos	66	bee SE 73%; wasp SE 68%	healthy sIgE neg	13	bee SP 71% wasp SP 100%	> mean control + 2 or 3 SD	SE not better w 3 Ag doses 19 % a-IgE neg,
138	Sabbah, 1998	bee 8 wasp 40 hornet 1	local reaction sytemic reaction ana shock	12 23 9	sLT SE 87%, BAT SE 100% ST SE 75%, SIgE SE 86% HR SE 91%	healthy sIgE neg	10	sLT SP 100%	sLT 250 pg/ml > bckg	SLT and BAT best coorelated to clinical history
100	Hipler, 1999	bee,wasp	bee/wasp allergics local reactions	84 6	bee sLT SE 93%: sIgE SE 87% wasp sLT SE 96%; sIgE SE 92%	healthy sIgE neg	10	bee sLT SP 67%; sIgE SP 70% wasp sLT SP 68%; sIGE SP 57%	nr	high reporducibility sLT not corr with severity
84	Szymanski, 1999	bee 19 wasp 18	allergic ST,sIgE pos pseudo ST,sIgE neg VIT failure	43 11 7	sLT maen ~5000 before IT sLT mean ~1500 after IT					sLT >600 in 10/11 patients pseudoallergic (ST,sIgE neg)
42	Marti, 1999	Culicoides mosquito	horses with summer eczema	12	10 sLT pos (SE 83 %)	healthy	16	sLT SP 100 %	> mean controls + 3 SD (359 pg/ml)	corr sLT / HR 0.95
105	SainteLaudy, 2002	bee,wasp hornet	local reaction systemic reactions ana shock	12 25 9	sLT SE 100%; BAT SE 100% ST SE 85%; HR SE 89 %	stung w/o reaction	10	sLT SP 100%	> 160 pg/ml a-IgE > 300 pg/ml	
38	Anliker,2003	bee 35 wasp 61	systemic reactions	96	sLT SE 92%; sIgE SE 70% ST SE 76%			controls from refs 58,59	3 SD> controls means	23 ST and sIgE neg : 20 CAST pos (87%)
99	Borelli,2003	bee 20 wasp 23		45	status after 3 yrs IT, test at 6,12, 24,36 mo, In 23, rise in ST treshold 16 field sting neg, all still CAST pos			>292 pg 0.01 ug bee > 239 pg 0.01 ug wasp		no value in monitoring IT
115	Scherer, 2005	bee 35 wasp 72 bee+wasp 43	anaphylaxis 143 local react 7	150	*	Non sensitized	40	**	***	****

* bee: SE SLT 92%, sIgE, 97%, sT 97%, BAT 93%
Wasp: SE sLT 90%, sIgE 88%, sT 97%, BAT 85%
** bee SP sLT 93%, sIgE 92%, ST 97%, BAT 94%
Wasp SP sLT 95%, sIgE 93%, ST 90%, BAT 95%
*** cutoffmeans + 3 SD and 100 pg > BcKg, S1> 2
**** Better discrimination between bee and wasp renom allergy with sLT and BAT than with ST or sIgE

Accordingly, the diagnostic gold standard in food allergy is usually considered to be the double blind placebo-controlled food challenge (DBPCFC) [31]. However, this procedure is quite cumbersome, expensive since usually it requires some hospitalisation, and may be quite dangerous for the patient. In addition, DBPCFC should be recognised not to be entirely reliable [32]. Accordingly, some in vitro test enabling to replace, at least in part, provocation food challenges would be most welcome.

Although theoretically the CAST assay could be of great interest in this indication, only three validated studies encompassing 151 patients and 79 controls are recorded [30,33] (Table 3). These studies show, however, that performance of in vitro food allergen-induced sLT release assays may be quite helpful in the diagnosis of food allergy. Particularly suggestive have been the recent results of Ballmer et al. [30] which show that CAST enables to distinguish between patients with immunological cross-reactivity between birch pollen, on the one hand, and celery, hazelnut and carrot, on the other hand, those presenting a clinical reactivity with positive DBPCFC from those who do not have been particularly suggestive.

It is obvious that the results are also very much dependent upon the quality and standardization of the allergen used [32], which is particularly difficult to achieve with food allergens. Accordingly, the use of recombinant food allergens [34] is to be encouraged and further investigated. It is also likely that combination of CAST with flow cytometric basophil activation assays will improve the diagnostic efficiency. Although sometimes useful on an individual basis [32,35], histamine release assays have often proven less reliable in food allergy; in particular, patients with food allergies often present with high spontaneous histamine release, making the interpretation of the test impossible [36].

Allergy to insect venoms

Several more or less anecdotal studies but at least 7 well controlled and validated studies in a total of 351 patients with bee and/or wasp venom allergies and 60 controls have been published (Table 4). Since diagnosis of IgE-mediated insect venom allergy by skin tests or determination of allergen-specific IgE is not entirely reliable [37-39], it appears that CAST should definitely take its place in routine diagnosis, at least in those patients where skin tests and/or specific IgE determinations do not match history. Up to 33% of insect venom allergy patients proven by provocation (insect sting) have negative skin tests [38,39]. An accurate diagnosis is particularly important to confirm the indication for immunotherapy [40]. In this area also, the combined CAST and basophil activation test appears to have a particularly high diagnostic efficiency [40,41].

In one field of veterinary allergy, the horse's summer dermatitis due to allergy to mosquito stings, the CAST has shown to be the most and only efficient diagnostic method [42].

Occupational allergies

Although individual cases with various occupational allergens have been occasionally reported, most of the published experience refers to latex (Table 5).

Among two controlled and validated studies, one showed for CAST a sensitivity lower than specific IgE determination and skin tests [43], the other one indicated an acceptable sensitivity (81%) and a particularly high specificity (97-100%) [44]. Considering that skin tests and specific IgE determinations are also not entirely reliable in latex allergy [45], cellular tests provide a useful diagnostic complementation, particularly in this occupational allergy which may have serious professional consequences. There too, combination of CAST with flowcytometry has proven particularly efficient (sensitivity: 93%; specificity: 100 %) [44].

Allergy to drugs

The availability of the CAST assay has since 1993 drawn quite a bit of interest, as with the exception of Betalactam allergy, skin tests and specific IgE determinations are seldom available or relevant. Furthermore, it soon appeared that CAST may also yield interesting results in some non-IgE mediated pseudo-allergies to drugs, such as NSAIDs [46-49]. However, in the first years, only anecdotal, not well controlled studies appeared (Table 6a) and it is only in the last few years that a large number of well controlled and validated studies has been published, particularly on Betalactams, NSAIDs and analgesics. We record at this time 21 validated studies encompassing 669 patients and 467 controls (Table 6b).

In immediate-type allergy to Betalactams, five studies [9,50-53] generally agree that sensitivity is rather low, varying between 30 and 50 %, depending also upon the type of Betalactam allergen used (drug itself or plurivalent drug-polylysine or drug-protein conjugate). Specificity, on the other hand, usually reached 80% or more. While that kind of sensitivity may well be considered too low for an ideal test, it is almost a matter of diagnostic philosophy to declare it useless on a routine or individual basis, as some have done [9]. Particularly considering that the other available tests (skin tests, specific IgE) are also far from being perfect [54], it may be considered, provided specificity remains high, that any positive CAST test in drug allergy contributes to confirm a diagnostic suspicion, while a negative CAST cannot exclude it. In particular, the CAST test has been found positive in numerous cases of Betalactam allergy proven by provocation challenge but demonstrating

Table 5. Occupational allergens; Latex.

Ref No	Author	Allergens	Patients	Nb	Result	Controls	Nb	Results	Positivity criteria	Comments
A. Anecdotal studies										
139	Cirla et al, 1995	wheat flour	allergic bakers	6	6/6 sLT pos					
102	SainteLaudy,1995	latex	latex allergics	4 3	sIgE pos 3/4 sLT pos sIgE neg 2/3 sLT pos	healthy	2	sLT neg	100 pg/ml > Bckg	HR least sensitive BAT pos also in HR a-IgE neg
B. Validated studies										
43	Marais et al, 1997	latex	latex allergics	23	sLT SE 43%; sIgE SE56% ST SE 86% -HR SE 45%	Healthy	10			patients with urticaria: sLT neg
44	Sanz et al,2003	latex	latex allergics ST pos	43	sLT SE 81%, sIgE SE 88%	Healthy	30	sLT 97-100%	sLT >300 pg/ml; SI >4 from ROC curves	

Table 6A. Drugs.

Ref No	Author	Allergens	Patients	Nb	Result	Controls	Nb	Results	Positivity criteria	Comments
A. Anecdotal Studies										
7,46	De Weck , 1993	Betalactams	allergic certain dubious	9 5	4/9 sLT pos 1/5 sLT pos					
140	Bircher , 1995	Betalactams	allergic	8	3/8 sLT pos					sLT pos in anaphylaxis only
113	Hippler , 1995	Betalactams Varia	allergic allergic	8 2	8/10 sLT pos	Healthy	2	2/2 sLZ neg		
47	Brunner,1996	ASA Analgesics	anaphylaxis anaphylaxis	9 6	sLT > in Prov pos	healthy	6			no HR to ASA
141	Miyahara,1996	Cefotiam	contact urticaria	3	3/3 sLT pos; 3/3 sIgET	Healthy	5	5/5 sLT neg		
71	Bircher ,1996	Betalactams	Anaphylaxis Urticaria Exanthema Dubious	5 6 5 6	5/5 sLT pos;3/5 sIgE; 5/5 ST 0/6 sLT pos; 2/6 sIgE; 5/6 ST 1/5 sLT pos;0/5 sIgE; 5/5 ST 0/6 sLT pos; 0/6 sIgE, 0/6 ST	Healthy	4	4/4 neg sLT, sIgE, ST		
142	SainteLaudy ,1996	ASA,benzoate dyes	allergics	27	12/27 pos (ASA 8/12;, benzaote 6/12, dyes 4 & 5 /12)					
48	Hippler,1997	Betalactams NSAIDs varia	allergics	14 6 10	sLT pos 10/14 sLT pos 4/6 sLT pos 6/10	Healthy	2	sLT neg 2/2		
143	Sabbah ,1997	Paracetamol	allergics	3	3 sLT pos					
144	Sabbah,1998	myorelaxants RCM	anaphylaxis	16	sLT > in cases of demonstrated anapyhlaxis					1 sLT pos to Gelofusin
49	Höxtermann ,1997	ASA	ASA intolerant	18	9 sLT pos.					
127	Leynadier, 1999	lidocaine	allergic?	1	sLT false pos					
128	Venière et al,2000	various drugs	allergic?	22	only 2 CAT and BAT pos				100 pg > Bckg	
129	Scherer,2003	Isosulfan blue	anaphylaxis	1	BAT pos, sLT neg					

Table 6B. Drugs.

Ref No	Author	Allergens	Patients	Nb	Result	Controls	Nb	Results	Positivity criteria	Comments
B. Validated studies										
56,57	Czech ,1995	ASA, C5a	ASA prov pos ASA prov neg	8 7	sLT C5a 1188 pg/ml (650-2100) sLT C5a 309 pg/ml (115-450)	Healthy	8	sLT C5a 376 pg/ml (120-400)		no sLT by ASA (10,100 ug/ml)
14	Wedi .,1996	ASA	ASA prov pos	6	sLT C5a > controls (~2000 pg/ml)	Healthy	46	sLT C5a ~200 pg/ml		no sLT by ASA - sLT produced by basos, not by eosinos
124	Mewes ,1996	ASA	Rhinitis,ASA prov pos Rhinitis ASA prov neg	8 9	sLT ASA 686 pg; C5a 2196 pg/ml sLT ASA 209 pg; C5a 751 pg/ml	Healthy	8	sLT ASA 346 pg;C5a 334 pg Bckg sLT 259 pg		no sLT > by C5a in controls ASA 10 ug/ml
145,146	Wolanczyk ,1997	ASA	Asthma ASA prov pos Asthma ASA prov neg	15 13	10/15 sLT ASA pos (SE 66%) 0/13 sLT ASA pos	Healthy	10	0/10 sLT ASA pos (SP 100%) sLT ASA < 50 pg/ml	sLT 200 pg/ml > Bckg	ASA 200,20,2 ug/ml
65	Czech., 1994	Additiva	Urticaria acute Urticaria in interval	8 7	sLT C5a, fMLP > patients in interval	Healthy	8			
147	Kubota, 1997	ASA Diclofenac Antipyrin	Urticaria,shock Rhinitis	8 12 5	sLT ASA SE 37%; mean 221 pg/ml sLT Diclo SE 50% sLT Antipyr SE 80%	Healthy Other drugs	5 5	sLT ASA SP 80%; mean 132 pg/ml sLT Diclo SP 90% sLT Antipyr SP 100%	sLT 100 pg/ml > Bckg SI > 2	
59,6	May 1999,2000	ASA, NSAIDs	Asthma prov pos Urticaria prov pos	22 16	sLT SE ASA 50%;+ NSAIDs 71% sLT SE ASA 81%;+ NSAIDs 100%	Healthy Atopic	30 20	SLT SP >99%	sLT > mean atopics +3SD	ASA+C5a > ASA alone but only in ASA intolerant 24 hrs blood storage OK
50	Bircher,2000	Betalactams	Anaphylaxis Urticaria Dubious allergy	7 10 10	sLT 6 pos sLT 0 pos sLT 0 pos				sLT 200 pg/ml > Bckg	sLT pos in anaphylaxis only
66	Busse,2000	ASA, benzoate metabisulfites	Urticaria,symptomatic Urticaria,asymptomatic	14 15	57% prov pos; 8 sLT C5a pos 20% prov pos; 6 sLT C5a pos	Healthy	15	Prov neg; sLT C5a neg		sLT C6a > by prov pos
55	Pierchalska,2000	ASA	Asthma,ASA prov pos Asthma,ASA prov neg	26 32	sLT Bckg 84 pg; ASA 127 pg/ml sLT Bckg 82 pg; ASA 122 pg/ml					ASA 18 ug/ml ASA > sLT in HI-60 cell line
58	Celik ,2001	ASA	Asthma ASA prov pos Asthma ASA prov neg	12 13	sLT Bckg 353 pg; ASA 453 pg sLT Bckg 354 pg; ASA 353 pg	Healthy	13	sLT Bckg 358 pg; ASA 405 pg		PGE2 inhibits sLT release
9	Lebel ,2001	Betalactams ASA Acetaminophen	sLT SE 43%	30 19 6	sLT SE 43 % sLT SE 21 % sLT SE 33 %	Healthy	64	sLT SP 79% sLT SP 88% sLT SP 100%	ratio net sLT / cutoff given by mean of controls (161, 152,285)	sLT better as HR but individual results considered not useful
148	Wedi ,2000	ASA, C5a	Urticaria, asthma ASA prov pos	18	sLT C5a SE 83%; 692 pg/ml	Healthy prov neg	66	sLT C5a SP 94%; 117 pg/ml	sLT C5a > 300 pg	also C5a > to fMLP , PAF
67	Worm , 2001	Additiva (Tartrazine 3 Benzoate 4 Nitrites 7,varia 4)	AD pos prov AD neg prov	9 9	sLT Ag SE 78%	Healthy	10	sLT Ag neg(< 100 pg/ml net)	sLT > mean controls + 3 SD	a-IgE non responders 0/28 sLT a-IgE : AD 2826 pg; controls 1689 pg
131	Abrahamsen,2001	C5a, a-IgE ASA	Asthma ASA prov pos Asthma ASA prov neg Allergic asthma (IgE)	10 12 7	sLT C5a 14.4 pg / 10x5 cells sLT C5a 22.9 pg / 10x5 cells sLT C5a 9.6 pg / 10x5 cells	Healthy	9	sLT C5a 7.5 pg / 10x5 cells	C5a does not distinguish ASA prov pos or neg asthmatics	C5a / a-IgE sLT ratio diagnostic for intrinsic asthma- sLT produced by basos
51,52	Sanz , 2000,2002	Betalactams	BP/AX ST pos AX ST pos BP/AX ST neg	23 34 16	sLT SE 35%; + BAT : SE 76 % sLT SE 32%; + BAT : SE 61% sLT SE 47%; + BAT: 47%	Healthy ST neg	30	sLT SP 83% BAT SP 93%	sLT 100 pg/ml > Bckg	optimal SE by combination sLT + BAT
53	Medrala, 2002	Betalactams	Immediate type allergy	33	sLT BP SE30% sLT PPLSE 18% Combi Ag 74 % sLT BPO-HSA SE 50%	Healthy ST neg	13			
64	Gamboa,2003	Dipyrone	Anaphylaxis Urticaria	15 11	sLT SE 52%; BAT SE 42 % sLT + BAT SE 76 %	Other allergies Prov neg	30	3 sLT pos ; SP 90% BAT SP 100%	sLT 100 pg/ml > Bckg; SI > 3	optimal SE by combination sLT + BAT +ST
149	Schäfer,1999	ASA	asthmatics, 10 AIA, 8 ATA	18	sLT >, PGE2 < in AIA	healthy	10	no > sLT		> sLT in AIA in vitro and in vivo (Provoc)
61	Sanz et al, 2003	ASA + 4 NSAIDs	Asthma Prov pos Urticaria	60	ASA sLT SE 22% 4 NSAIDs SE 48%	Heallthy Prov neg	30	ASA sLT SP 89% 4 NSAIDs SP 68%	sLT 100 pg/ml > Bckg SI>2	Prov pos within 1 month: SE 88%
110	Faria,2003	ASA	asthma to ASA urticaria to ASA	15 14	only 1/15 sLT pos 14 sLT neg	atopics healthy	9 3	2/9 sLT pos 3 söT neg	sLT 180 pg > bckg	CAST not useful
150	Pereira-Santos, 200	ASA, Dic	urticaria to NSAIDs	37	4 sLT pos/6 Prov pos Prov neg = sLT neg	resp allergy healthy	20 15	all sLT neg all sLT neg		corr CAST pos/ASA prov pos p=< 0.01

negative skin and specific IgE tests [52]. Taken on an individual patient's basis, CAST may therefore often provide some useful information.

In the case of pseudoallergic reactions to aspirin and other NSAIDs, no other diagnostic in vitro test has been available before CAST. Among the ten controlled and validated studies published so far (Table 6b), one is completely negative [55], one because of a sensitivity of only 21% despite a specificity of 88% leads to uselessness [9] and the other eight studies report on diagnostic efficacy, with sensitivities varying between 60 and 71% and specificities between 97 and 100%. However, three of these studies report positive results only with C5a and not with ASA [56-58] and two report that use of other NSAIDs beyond ASA alone considerably improves sensitivity [59,60]. Heterogeneity in the allergens used, particularly in the dose, may possibly explain the heterogeneity in results; for example, the only totally negative study [55] used a single dose of ASA almost 20-50 times lower than most other studies. In the case of pseudoallergy to NSAIDs also, combination of CAST with flowcytometry may improve diagnostic efficiency [61]. In any case, the classical statement often repeated over 20 years [62] that no diagnostic in vitro tests exist in pseudoallergy to NSAIDs is certainly no longer valid. Our point of view is reinforced by the recent report that increased release of 15-HETE by leukocytes upon incubation by ASA is also detected in asthmatics intolerant to aspirin [63].

Another area in which CAST has recently shown to be useful is in IgE-mediated allergy to analgesics, e.g. Dipyrone (Metamizol) [64].

Finally, a few studies point to the usefulness of CAST in allergic or pseudoallergic reactions to food additives such as dyes or preservatives [65-67]. Such reactions are relatively rare and difficult to confirm clinically by provocation tests, because of the usual multiplicity of suspected substances. However, the frequent success of avoidance diets in chronic urticaria (68) indicates that additives may exhibit causal or at least adjuvant effects more often than usually believed. Since CAST shows sometimes positive results and has a high specificity, and since no other tests are reliable, it seems that the CAST test would be worth carrying out before provocation tests.

In summary, despite a suboptimal sensitivity, the CAST test has definitely shown to be helpful in diagnosis of immediate-type allergies and pseudo-allergies to drugs, particularly when skin tests or determination of drug-specific IgE are not available. In delayed-type clinical reactions, on the other hand, e.g. morbilliform exanthema, which are caused by drug-specific T lymphocytes [69,70], the CAST assay is mostly negative [71].

Immunotherapy follow up

Immunotherapy with aeroallergens for allergic asthma and/or rhinitis has been shown by many studies to have beneficial effects on clinical symptoms of allergy [72]. Its mechanisms of action are still the object of discussion and hypotheses. Some of the major immunological effects are a shift in the balance of allergen-specific Th1 and Th2 lymphocytes and a stimulation of T regulator lymphocytes, with corresponding changes in the pattern of allergen-induced cytokine production [73]. The diminution of allergic symptoms upon allergen exposure, however, is probably more directly related to an impaired release of inflammatory mediators, such as histamine and sulfidoleukotrienes, that are responsible for these symptoms. Indeed, several authors have reported a decrease of allergen-induced histamine release from blood basophils following immunotherapy [74].

It was therefore logic to investigate with the CAST assay whether the beneficial effects of classical immunotherapy, which are often demonstrated by more or less subjective criteria, can be effectively and objectively confirmed by an in vitro test.

Several studies (Table 7) have attempted to answer that question, particularly in the field of inhalation allergy to house dust mites and to pollens [75-78] and insect venoms [79-84]. In insect venom allergy, although a diminution of sLT release is sometimes observed following immunotherapy, the correlation between in vitro results and clinical benefit is not impressive [83] and monitoring of venom immunotherapy usually considered not useful. In inhalation allergies, when clinical symptoms have decreased by more than 50% following immunotherapy, a corresponding decrease in SLT release in vitro is consistently observed [75,78]. The most impressive results reported to date are those following immunotherapy combined with anti-IgE therapy [85]. In that case, the diminution of allergen-induced sLT release in vitro was highly significant and correlated with clinical benefit.

More follow up studies, possibly with more sophisticated techniques (e.g. with inclusion of allergen dose-response curves, repeated tests at 3 months intervals, etc.) to document the pathophysiological success of immunotherapy regimens would certainly be desirable.

Drugs and biological factors affecting sLT production

In a few published studies CAST has shown (Table 8 A) to be suitable for pharmacological studies of drugs affecting basophil reactivity and mediator release, such as antihistamines [21], steroids [86,87], ambroxol [88] or various chemicals [89]. In pharmacological research on potential anti-inflammatory drugs, particularly in drugs affecting leukotriene production, the CAST assay

Table 7. Followup Immunotherapy.

Ref No	Author	Allergens	Patients	Nb	Result	Controls	Nb	Results	Comments
117	Cosachov,1995	GP,ragweed Moulds	Asthma atopics	6	sLT diminished from 561 pg/ml to 196 pg/ml after 6 months IT (group mean)				
75	De Weck , 1995	HDM	IT HDM IT Placebo	16 14	sLT dim > 50 % in 50 % of patients IT 12 months no change sLT in placebo treated				
139	Cirla, , 1995	wheat flour	IT	6	sLT Ag 558/ml pg to 171pg/ml after 3 months IT sLT a-IgE 2141 pg/ml to 1416 pg/ml				
76	Ferrer , 1998	HDM GP HDM + GP	allergics IT 2.5 yrs allergics IT 2.5 yrs	35	sLT HDM 1320 pg/ml sLT GP 1884 pg/ml	allergics no IT allergics no IT	57 15	sLT HDM 2028 pg/ml sLT GP 2213 pg/ml	sLT a-IgE with IT 2086 pg/ml sLT a-IgE no IT 1339 pg/ml
79	Busse , 1996	venoms							
80	Jutel , 1996	venoms	Ultra-rush IT (3-6 hrs)	7	no < sLT but < total HR				no obvious decrease sLT
81	Eberlein, 1997	venoms							
77	Medrala,1997	GP	IT clin success IT clin failure	8 9	sLT GP before IT 501 pg,after IT 386 pg, after season 615 pg/ml sLT GP before IT 824 pg,after IT 1057 pg, after season 797 pg/ml				decrease sLT only in patients clinically reacting to IT
78	Saraçlar,1997	HDM	allergics under IT (12 mo)	13	sLT < 50% in 5/6 patients with symptoms < 50% after OZ	allergics, no IT	5	sLT unchanged	< nasal prov sneezes 53% after IT
82	Pierkes,1999	wasp venom	systemic allergy ST pos, sIgE pos	22	< sLT and HR after rush IT (1 week)				< sLT due to IL-10 , IFN-gamma
83,99	Borelli, 1999,2003	bee,wasp venoms	bee allergics wasp allergics	20 23	after 3 yrs IT: 23 ST diminished, only 2 CAST become neg 16 provoc. neg after IT; all remain CAST pos				CAST not useful to predict outcome of IT
84	Szymanski, 1999	bee 19 wasp 18	allergic ST,sIgE pos pseudo ST,sIgE neg VIT failure	43 11 7	sLT maen ~5000 before IT sLT mean ~1500 after IT				sLT >600 in 10/11 patients pseudoallergic (ST,sIgE neg)
85	Kopp,2002	GP Birch	rhinitis (6-17 yrs)	23 23	IT GP + anti-IgE Ab : sLT 416 pg/ml IT birch + anti-IgE Ab : sLT 207 pg/ml	GP IT alone Birch IT alone	24 22	sLT 2490 pg/ml sLT 2489 pg/ml	IT < sLT also to Ag not used for IT

may be quite useful, but up to now such results from the pharmacological industry have rarely been published.

In the literature, a number of isolated studies document the effect of biological factors such as cytokines (90-93) on leukotriene release. Antibodies, such as secretory IgA [94] and particularly anti-IgE and/or anti-FcER1 antibodies, as encountered in many cases of chronic idiopathic urticaria, have been shown to induce sLT production [95,96]. Since some of these antibodies do not induce symptoms and mediator release, the CAST assay with autologous serum could become an important diagnostic tool in chronic urticaria.

Correlation of CAST with clinical reactivity and other diagnostic tests

From a practical point of view, it is important to evaluate the diagnostic value of CAST in respect to the clinical allergic symptoms and to the other diagnostic tests available such as skin tests, specific IgE determination (e.g. CAP assay) and other cellular tests.

Relationship to nature and severity of the clinical allergic reaction

Since CAST detects the capacity of basophils to release sLTs as mediators of allergic inflammation, thereby expressing cellular reactivity [7,97], which is not obligatorily proportional to sensitisation manifested by the presence of IgE antibodies, one could theoretically expect better correlation of CAST with the severity of clinical symptoms. However, there are not many data available on this topic.

In allergic rhinitis, a good correlation between CAST results and the severity of reaction to allergen challenge provocation has been reported [21]. In asthma, on the other hand, correlation between asthma severity and the level of CAST positivity in sLT pg/ml is rather poor [7,98]. The same has been reported for in hymenoptera venom allergy [99,100]. In allergic clinical reactions involving primarily local tissue

Table 8. Effect of various factors on sLT production.

Ref	Author	Allergen	Drug	Patients		Effect	Comments
A. Drugs							
21	Kalayci ,1995	HDM	cetirizine 10 mg/d, 7 days	Rhinitis	12	< sLT ~40% by Ag, not by anti-IgE	no change sLT after placebo ttt (cross-over)
86	Crocker ,1995		fomioterla anti-sLT	allergics		no effect of Beta agonist on sLT release inhibition of anti-sLT on SLT release	
87	Crocker ,1997	GP,HDM	steroids	Allergics	10	No effect of steroids in vitro on spontaneous sLT release but < sLT in IL3-primed cells challenged by ionomycin or Ag	> spontaneous sLT release in season
88	Gibbs ,1999	ConA, 48/80	ambroxol	healthy blood donors	04-jul	< sLT, HR IL-4, IL-13 from basophils and mast cells by > 50%	more efficient inhibition after cell princubation for 5-15 min
89	Strenzke ,2001		HgCl2			increase sLT induced by	
B. Biologics							
90	Jankowska ,1995		IFN gamma			< sLT relese	
91	Krasnowaska ,2000	various Ag	IFN	Pollinosis Allergic asthma	15 23	< sLT after 15 min preinc with IFN	
92	Kraus-Filarska,1998		LPS Klebsiella	allergic asthma COPD	19 9	increased sLT; 462 pg/ml not significant increase sLT; 474 pg/ml	10 healthy controls :sLT 191 pg/ml asthmatics > sLT to LPS
151	Taskila, 2000	nettle	contact urticaria				
94	Iikura , 1998		secretory IgA	healthy donors	9	sLT , HR by immobilized sIgA	priming effect of IL-3 at 5-50 pg/ml
93	Wehner,2001		Staph.aureus enterotoxins	atopic dermatitis sIgE Staph pos	15	Bckg : 7 pg; IL-3 156 pg : Staph 107 pg/ml Staph + IL-3 : 2802 pg/ml	9 healthy : Bckg 7 pg; IL3 7 pg: Staph 17 pg Staph + IL-3: 17 pg - sIgE Staph neg
95	Wedi ., 2002		autologous serum	chronic urticaria,ST pos chronic urticaria,ST neg	20 20	increased sLT to autologous serum in individual cases of both groups sLT correlated with urticaria severity	10 healthy, 10 atopic controls, no sLT sLT inhibited by dapsone, chloroquine lidocaine, mizolastine
96	Ferrer , 2002		autologous serum	chronic urticaria	60	some sera cause sLT , HR	50 controls, no sLT

mast cells, it is understandable that a test reflecting the reactivity of blood basophils may not be closely correlated. This might be different in generalized allergic reactions, such as anaphylactic shock. Indeed, it has been reported in Betalactam allergy that CAST is positive only in cases of anaphylaxis [50,71] but this has not been confirmed by other authors, who find CAST positive also in cases of generalized urticaria [51,52]. A correlation of CAST with anaphylaxis has also been reported for foods [33]. There is evidence that sLTs are released in vivo during anaphylactic reactions [101].

Correlation with other cellular tests

The histamine release test has been used in allergy research since over 40 years but has seldom been applied routinely to allergy diagnosis (2) mainly because it is relatively cumbersome and not very sensitive. In principle, however, it is quite similar to the CAST assay. Both tests have been compared by several authors in clinical situations, particularly for inhalant allergies [98,102,103], insect venom allergies [37,104,105], food allergies [33,34,36,106] as well as hypersensitivity to NSAIDs [57,102] and other drugs [63,66]. For anti-IgE stimulant and protein allergens, correlation has usually been found to be rather good but this has not been the case for haptens and drugs (Fig 1). In general, histamine release has been found to be less sensitive than CAST [50]. An additional drawback is that histamine release may be non specifically high and false positive, in case of allergen cytotoxicity and/or of recent in vivo allergen exposure [36].

There are a number of situations (e.g. drugs, non specific reactions to some basophil stimulants) in which histamine and sLT release may be dissociated, since both rest on different intracellular mechanisms [61,107]. Dissociation between histamine and sLT release has also been observed on bee venom allergy [37,104,108].

Correlation with skin tests

For most protein allergens such as inhalants [7,18,98] and insect venoms [37,104,105, 107], there is a quantitative correlation between skin tests and CAST results but this correlation is usually not very high, in the range of r= 0.4 – 0.6. It has also been reported that a number of patients allergic to drugs [51,52], insect venoms [108,109] or latex [43] may show positive CAST despite a negative skin test. In a number of instances, e.g. hypersensitivity to NSAIDs and non-IgE mediated allergies, for which no skin tests exist, CAST offers a valuable in vitro approach to diagnosis. In such cases, a positive CAST result may be meaningful but a negative result never permits to exclude allergy or hypersensitivity.

Correlation with allergen-specific IgE

In all instances where basophil reactivity and mediator release to allergens in vitro is based on the presence of cell-bound specific IgE, a correlation with the detection of allergen-specific serum antibodies (e.g. CAP assay) should be expected. Indeed, in some instances, particularly in inhalation allergies to protein allergens, such correlation has been found. The quantitative correlation, however, is not very high in most instances (r = 0.20 – 0.72). While the presence of allergen-specific serum IgE merely reflects sensitisation, basophil mediator release reflects additional parameters such as IgE avidity and cellular reactivity [7,15].

In several instances, particularly in allergy to Betalactam antibiotics, determination of allergen-specific serum IgE has been found to be less sensitive than CAST [51,52].

Complementarity of CAST with flow cytometric basophil activation

As already indicated, the combination of sLT determination by ELISA and of basophil activation by flowcytometry, as commercially available in the CAST Combi test, may be of great diagnostic benefit, as already validated for Betalactam [51,52], NSAIDS [61], metamizol [64] and latex [110] allergy. Following a single incubation of buffy coat leukocytes stimulated by allergens in vitro, this test combines the flowcytometric analysis of CD63 expression on basophils with the determination of sLTs by ELISA on the cell supernatant.

One could have thought that both tests would run in parallel and show high correlation. As a matter of fact this is true for high levels of hypersensitivity and protein allergens (Fig. 1). But it is not quite true for weak levels of stimulation, as frequently encountered with drugs. In that case, the combined performance of both tests markedly increases sensitivity without significantly diminishing specificity (Fig.1). The correlation between FAST and CAST becomes markedly poorer with drugs stimulating basophils by non specific mechanisms, like NSAIDs. For allergies and pseudoallergies to drugs, as well as for food allergies, the combined test is certainly recommended.

Technical considerations affecting the interpretation of results

A mere review of published results might be misleading if a number of technical factors which may affect the results were not discussed. Indeed, as is apparent from the literature and from personal experience, a number of technical factors may be responsible for discrepant results and many publications, unfortunately, are not sufficiently explicit in that respect.

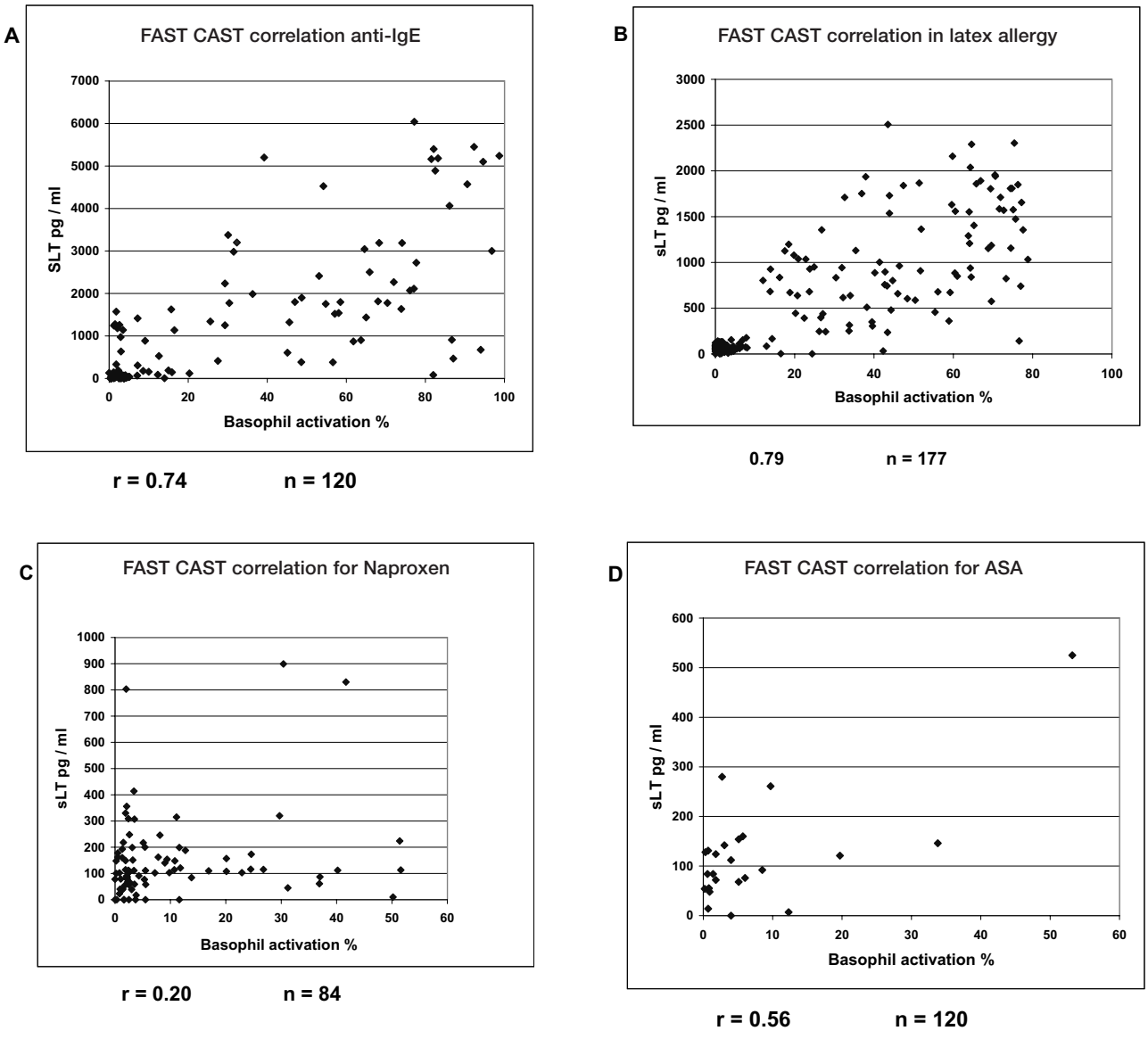

Figure 1. Correlations between FAST (basophil activation) and CAST (pg SLT/ml) for positive anti IgE controls (panel A), allergy to Latex (panel B), hypersensitivity reactions to Naproxen (panel C) and hypersensitivity reactions to acetylsalicylic acid ASA (panel D).

Various technical aspects have been discussed elsewhere [7, 8,15]. The good inter- and intra-assay reproducibility has been confirmed by several authors [19,25,36,111,112].

Allergen dose

With most protein allergens (e.g. pollen house dust mites, foods), the allergen doses found to yield positive results in allergic patients vary between 1 and 100 nanograms protein/ ml [7,15,18,19,98]. Since most allergens used are not standardized in terms of their contents in molecularly defined allergens, quantitative comparisons are indeed difficult, particularly, as may be the case for immunotherapy follow up, when the test is repeated at different times with different allergen batches. On the basis of histamine release assays, which typically show a bell shaped dose response curve, it has been argued [12] that the use in routine testing of a single allergen dose in CAST is not acceptable. This may be true if absolute quantitative comparisons were required but in terms of segregation between positive or negative CAST response, experience has shown that a single dose of 10-20 ng protein almost always enables to discriminate, at least for aeroallergens and foods. For

most protein allergens, the CAST dose-response curve has a very broad bell shape over 3-4 potencies [15,113]. Accordingly, and in contrast to what is stated in a recent position paper [12], a single concentration of allergen (usually 1 – 100 ng/ml final concentration) will suffice to distinguish between negative and positive cases. For insect venoms, on the other hand, which contains substances causing non specific basophil stimulation at higher doses in healthy controls, the use of at least two allergen concentrations is beneficial [37,104], although not essential [99]. In drug allergy also, the routine use of two allergen doses, usually in a ratio of 1:5 or 1:10 has been found to increase sensitivity [52,61,64]. The problem of optimal allergen concentrations for CAST will probably become simplified by the future increased use of recombinant allergens [34].

Spontaneous sLT production

In the CAST assay, nonstimulated leukocytes used as negative control, seldom yield more than 200 pg sLT / ml, usually markedly less, although some authors report as much as 17% of random patients showing a non stimulated background of > 300 pg sLT / ml (114). Consistently higher negative controls in an experimental series should raise the suspicion of some technical mishap, such as the use of endotoxin-contaminated water, of non tissue culture grade plastic tubes for incubation or performance of the test in the open air and not in airflow sterile hoods (accidental contamination by aeroallergens!). Such disturbing factors are usually easy to identify.

The interpretation of data from individual patients showing an elevated non-stimulated control is more difficult. Such instances have been observed with variable frequency; i.e. l 200 – 300 pg/ml in 5-10% in some series [61,115] but as much as 17.9% [28/145] with >300 g/ml in others [114]. The usual interpretation of such findings is that patient basophils have been stimulated in vivo shortly before blood sampling by exposure to allergen(s) or to some endogenous inflammatory agent (e.g. infection). The increase of spontaneous sLT production (and /or histamine release) ex vivo during and shortly after the pollen season [18,19,116] or in food allergy where hidden allergen exposure and cross reactivities are probably frequent [33,36] are well documented. Even in cases with an abnormally high spontaneous sLT production and negative control, interpretation of the test remains possible if the sLT released upon stimulation by the putative allergen is markedly higher (stimulation index > 2 or 3).

Occurrence of non releasers

It is essential to perform also a positive control, to ensure that the cells used are viable and that the stimulation conditions permit the production of SLT. As well known and documented, the releasability of mediators by basophils is eminently variable [7,117] and influenced by many factors [97,118]. As standard, IgE-related basophil activation stimulants, anti-IgE antibodies and later anti-IgE Fc R_I antibodies have been used in the CAST assay as positive controls [7,13]. Nevertheless, some patients behave as “non releasers” and the negative data obtained upon allergen stimulation are then difficult or impossible to interpret. More questionable are positive results obtained with some putative allergen or ligand, while the anti-IgE induced control stimulation remains negative. With the histamine release assay, the percentage of non releasers may be quite sizeable in some studies: up to 20 – 25 % [2,36]. In CAST, the percentage of non releasers with anti-IgE was usually much lower, below 10% [55,58] and even below 3-5% when using anti-IgE FcR_I [7,26,99,115], although some series report as much as 19% of the patients releasing less than 500 pg sLT /ml [114].

Other non specific basophil stimulants may be used as positive controls, such as fMLP, ionomycin or concanavalin A. These stimulants, however, are non specific, often also act on other cells than basophils and test stimulation cellular pathways which are different from those triggered by IgE. They are therefore, theoretically at least, not strictly controls for IgE-mediated allergen stimulation but merely a cell functionality test.

Positivity criteria

A major problem in comparing and interpreting data from the literature is that positivity criteria used by various authors, including those recommended by the manufacturers, have often been quite different and have varied with time. Quite frequently, results of allergen stimulation have been considered positive when indicating 200 pg sLT/ ml above the negative control (or background) [104,119] but for weak stimulations, such as with drugs, particularly NSAIDs, the optimal cutoff to achieve optimal sensitivity and specificity has been 100 pg/ml above background [52,64]. However, in order to account for cases of relatively high background, an additional criterion has been recommended, namely a stimulation index (specific stimulation / negative control) higher than 2. Lately, optimal cutoffs have been checked for most allergens by ROC curves [51].

Another way to define positivity has been to set up the mean of sLT pg/ml induced by allergen in a group of clinically negative controls and to accept as positive values those exceeding 2 or 3 SD from that mean [37,99,104,112]. Since, particularly with drugs, the mean of the control group may be quite variable, this mode of calculation was found to be rather complicated and sometimes misleading.

In any case, it is quite important that published CAST studies in the future clearly indicate the positivity criteria used.

Role of chronic allergen exposure

Theoretically, it could be expected that chronic allergen exposure may affect the results of cellular tests, in particular CAST, in two ways. On the one hand, basophils stimulated in vivo by allergens may continue to release sLT apparently spontaneously in vitro and cause high negative controls. Indeed, this has been observed in grass pollen allergics during the pollen season [18,116] despite a recent contradictory report using the FAST test [120]. Another possible effect of chronic allergen exposure is to render basophils non specifically hyperreactive to various stimulants, as observed also in atopic dermatitis and in house dust mite allergy. Such an effect appears to be the rule in NSAID hypersensitivity, where the cause for basic hyperreactivity is probably some endogenous pro-inflammatory affection [61]. But it has also been observed in reactivity to C5a: patients with latex allergy or drug allergies are in part hyperreactive to C5a, which is not at all the case for insect venom allergics (T. Jermann, unpublished). The formal proof that this hyperreactivity is linked to chronic allergen exposure, however, is still missing.

Role of IL-3 priming

The fact that priming or simultaneous addition of IL-3 considerably increases the sLT release induced by allergens has been reported more than 15 years ago [97,118] and has been used since the beginning in the elaboration of the CAST assays as used in routine diagnostic practice [7]. The practical importance of adding IL-3 to obtain adequate sensitivity has been confirmed [26,121]. The potentiating effect of IL-3 seems more intense for sLT production than for histamine release. However, there has also been some opposition, claiming that IL-3 alone, even at the low concentration recommended [122,123] may stimulate basophils and cause false positive, non specific reactions. This controversy has been recently revived, suggesting, for example that the addition of 2 ng/ml IL-3 increases by 70% the degree of background basophil activation [123]. While it may be true that the addition of IL-3 slightly increases the background, this comes up on a very low level and is not significant in view of the positivity criteria usually chosen. In any case, particularly in drug hypersensitivity, the addition of IL-3 often appears essential for obtaining adequate sensitivity and does not seem to affect specificity. Basophils from atopic donors seem more sensitive to IL-3 priming than normals [15,119].

Role of C5a activation

C5a is a well known non specific activator of basophils [97] and low non stimulating concentrations of C5a have been thought to enhance the stimulation induced by specific allergens [59,60]. This has enticed some authors to use C5a, either alone or together with allergens, to perform diagnostic CAST assays [48,56,57,110,113].

In fact, it does not appear that C5a at concentrations of 10^{-8} M and below, really augments the reactivity of normal "healthy" basophils [59,124]. This may be different, however, when the cells already possess, to start with, some state of non specific hyperreactivity, which may be the case in patients with chronic urticaria [125], severe atopic dermatitis [126], asthma and particularly hypersensitivity to NSAIDs [56,57,61,110]. In such cases, C5a alone at the concentration of 10^{-8}M, may already induce basophil activation and sLT release [49,57,110,127-129]. At the higher concentration of 10^{-7} M. in various categories of allergic patients, C5a alone induces significant sLT release in about 50% of the cases (T. Jermann, unpublished).

The general recommendation at present is not to use C5a as an "adjuvant" but it may be interesting to establish, from a C5a dose-response curve, whether the patient possesses hyperreactive circulating basophils at the time of blood sampling.

Cellular origin of sLT in blood tests

SLTs may be produced by different circulating blood cell type, basophils, eosinophils, monocytes and platelets [130] and therefore it may be asked which cells are contributing to the sLT release induced by allergens in whole blood or in isolated leukocytes. The capacity to produce sLT, when induced by a non specific stimulant such as ionophore (A 12387) or fMLP, is about 100-fold lower for eosinophils than for basophils or monocytes. Experiments performed on isolated basophils and /or on basophil-depleted cells have clearly shown that the main providers of sLT upon IgE-mediated blood cell activation are the basophils, to an extent of more than 90% [97]. This seems also to be the case in hypersensitivity to NSAIDs, which is not mediated by specific IgE [131].

Use of passive sensitisation with serum

Since the induction of sLT release by basophils from allergic patients implies interaction of allergens with specific IgE on the cell surface, it may be expected that the same IgE antibodies, when passively transferred to normal basophils, would also cause sLT release in the presence of allergens. This has indeed been used with CAST, e.g. using sera from food [36] or drug [132] allergic patients and a mixture of normal donor basophils. This has some potential practical applications, since it is not always possible to send in due time the patient's leukocytes to the laboratory performing the CAST assay. However, it seems that

this method is less sensitive than when using patient cells. In a group of food allergic patients, the CAST tests performed after passive sensitisation was only positive when the serum used contained at least 3.5 kU IgE/L of specific IgE [36]. But the passive sensitisation method can also be used across some species, e.g. to detect dog IgE with human basophils [133].

Effects of blood storage

The need to use fresh blood or isolated leukocytes is the main obstacle to the routine use of cellular tests in allergy diagnosis. Investigations on the possibility to keep the blood for several hours or days after blood sampling have shown that the optimal conditions for keeping or sending the blood are the following: 24 hours at room temperature, suspension in ACD medium. Under such circumstances, performance is almost similar to that of fresh cells [7,15,36].

Avoidance of drug therapy before blood sampling

Several groups of drugs are known to impair basophil reactivity and mediator release in vitro and in vivo. Some antihistamines have been shown to impair the reactivity of ex vivo basophils [21]. Accordingly, it is usually recommended that patients should avoid to take antihistamines for at least 3 days (4 weeks in the case of astemizol) before blood sampling in view of a CAST assay.

For corticosteroids, the recommendations should be more differentiated. Unfortunately, the data available are insufficient. This matter is important in practice, since many severely allergic patients, particularly asthmatics under steroid therapy, cannot interrupt treatment merely for a diagnostic test. Glucocorticoids in vitro have been reported to indeed inhibit allergen- or non specific stimulant- induced basophil activation and mediator release in a dose-dependent manner [21]. Orally administered steroids appear to interfere with ex vivo histamine and/or sLT release [86] but the time required for full recovery of basophil reactivity after treatment interruption is not precisely known. It is usually recommended to abstain from oral steroid therapy for 8 days before blood sampling, but this may not always be possible. The effect of oral steroids on CAST is probably also dependent upon the dose and duration of steroid therapy. Despite oral steroid therapy, 15 from 22 patients still had positive CAST tests to NSAIDs [59].

For steroids administered by inhalation, which is the case for a large majority of patients with allergic rhinosinusitis or asthma, the evidence that treatment could interfere with the CAST assay is not obvious [55]. In some studies, it appeared that the duration of the steroid effect on ex vivo basophil mediator release was about 14 days [59]. A more formal investigation on the effect of inhaled steroids on sLT release, however, could not be found in the current literature.

Conclusions

During the past 10 years, the CAST assay has been used in allergy diagnosis in a variety of indications, such as inhalation allergies, allergies to insect venoms, foods, occupational allergens and various drugs. A large number of reports on CAST diagnostic value, however, have been anecdotal. A meta-analysis of validated and well controlled studies encompasses 37 studies, 1614 patients and 1145 controls. This should definitely establish the value of this diagnostic test, particularly in cases where other in vitro or in vivo diagnostic tests are not reliable, such as food or drug allergies, as well as in non-IgE-mediated immediate hypersensitivity reactions.

However, a number of questions about the CAST diagnostic assay are still open or have not been systematically explored. This may explain, in addition to the practical limitations inherent to all allergy cellular tests, why CAST has not yet become a very widely used assay worldwide, having gained broad acceptance in some countries but not in others.

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