

Evaluation of basophil activation in food allergy: present and future applications

Wayne G. Shreffler

Purpose of review

The diagnosis of immediate hypersensitivity relies on specific IgE and history. Because of low specificity, however, provocation challenges are often necessary. Furthermore, IgE testing does not predict features such as reaction severity; nor can it discriminate cross-reactivity from multiple sensitizations. Direct and passive basophil activation tests may address these needs. In addition, measuring basophil activation *ex vivo* may be useful for monitoring patients with food allergies.

Recent findings

Several papers using basophil activation tests demonstrate comparable sensitivity and specificity to current testing for food allergy. Flow-based basophil activation tests have also been used to assess functional characteristics of patient IgE. Finally, several activation phenotypes have been identified as markers of allergic inflammation *in vivo*; these phenotypes appear to correspond to earlier reports of spontaneous histamine-releasing basophils in patients with active allergic inflammation.

Summary

Although in their early stages, direct basophil activation tests may prove to be useful in the clinic. Indirect basophil activation studies are useful when applied to compare functional aspects of IgE. Identification of basophil activation *ex vivo* is a promising approach for monitoring allergic inflammation.

Keywords

basophil, CD63, CD203c, food allergy

Introduction

Basophils are important effector cells for allergic disease. This review focuses on what is currently known about basophil activation and the phenotypes that can be readily measured and have potential diagnostic or prognostic utility in food allergy. There are three contexts discussed here in which the measurement of basophil activation may be useful: first, direct stimulation of basophils from patients as an assay for immediate hypersensitivity; second, indirect stimulation of basophils that have been passively sensitized as a functional assay of IgE; and third, assessment of patient allergic inflammation by measurement of basophil activation status *ex vivo*.

Background

Although basophils are the least abundant circulating leukocyte, in IgE-sensitized individuals they represent the largest population of antigen-specific cells in the circulation. The biological implications of this are myriad: basophils are known to migrate rapidly into sites of inflammation, where they can release a wide variety of chemokines, cytokines and other inflammatory mediators [1]. There, they are likely to be responsible for the majority of early local IL-4 (interleukin-4) and IL-13 production [2], which may have important implications for the adaptive immune response [3]. The diagnostic and research potential of this fact have been recognized for more than a decade. Because basophils are circulating leukocytes, they are readily accessible. In addition, because they can be stimulated *ex vivo*, they provide the theoretical potential of measuring more than specific IgE, but rather a biological response from cells that were, until shortly before the assay, subject to the host's (our patient's) milieu.

The earliest measurements of basophil activation *ex vivo* were by the release of histamine or other mediators, for example leukotriene C₄ (LTC₄). Due to the need for substantial enrichment of basophils, this method has been used almost exclusively for functional studies of IgE–allergen interactions, often with passively sensitized cells, and has not lent itself to routine diagnostic use. In addition, in clinical scenarios of ongoing exposure to allergen, there is the problem of constitutive histamine release (see below) [4–6].

Flow cytometry is ideally suited for measurement of cellular subsets without fractionation. Pioneering efforts

Curr Opin Allergy Clin Immunol 6:226–233. © 2006 Lippincott Williams & Wilkins.

Mount Sinai School of Medicine, Division of Pediatric Allergy & Immunology, Department of Pediatrics, New York, USA

Correspondence to Dr Wayne Shreffler, MD, PhD, Mount Sinai School of Medicine, Division of Pediatric Allergy & Immunology, Department of Pediatrics, Box 1198, 1 Gustave L Levy Place, New York, NY 10029, USA
Tel: +1 212 241 7244; fax: +1 212 426 1902;
e-mail: wayne.shreffler@mssm.edu

Current Opinion in Allergy and Clinical Immunology 2006, 6:226–233

Abbreviations

BAT	basophil activation test
IL-4	interleukin-4
LAMP	lysosomal-associated membrane glycoprotein
LTC₄	leukotriene C ₄
PST	prick skin test

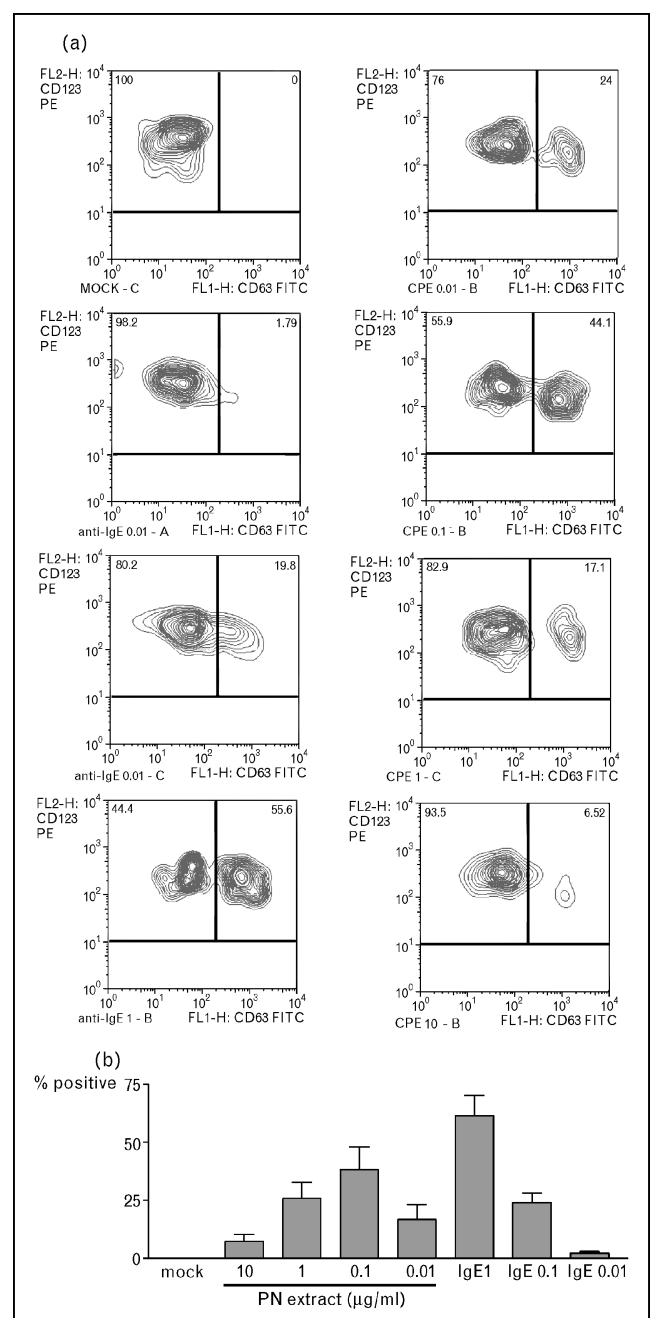
© 2006 Lippincott Williams & Wilkins
1528-4050

to measure basophil activation by flow cytometry were based on metachromatic dyes, such as Basic Orange 21 and Alcian Blue, to both detect and measure degranulation as a loss of signal [7]. These early methods were insensitive, however, due in part to the inherently poor sensitivity of measuring small decreases, versus increases, in signal.

With the discovery of the 53 kDa tetraspanin, CD63 [8,9], and the recognition that this secretory granule-associated protein is up-regulated to the cell surface concomitant with cellular degranulation, the stage was set for applying the detection of plasma membrane CD63 [also known as lysosomal-associated membrane glycoprotein-3 (LAMP-3)] to the detection of basophil activation. In 1991, Knol *et al.* [10] did just that in an elegant paper that first described the use of CD63 for measuring basophil activation. These authors carefully demonstrated the quantitative and kinetic correlation of CD63 and histamine from basophils stimulated both by FcεRI cross-linking and by the bacterial peptide, formyl-methionyl-leucyl-phenylalanine. They then showed by electron microscopy/immunogold staining that CD63 is translocated from the membrane of secretory granules to the plasma membrane.

There has been a report of poor correlation of CD63 and histamine under non-physiological conditions in a very careful paper on the question of degranulation as an 'all-or-nothing' event [11]; these authors concluded that basophil degranulation is a graded, not binary, response. Two findings from this paper regarding the relationship between CD63 up-regulation and histamine release are notable. One is that the percentage of cells clearly expressing CD63 did not equal the percentage of total histamine release. This is direct evidence against the binary model of histamine release, although not against CD63 as a marker of degranulation *per se*, as there are other pools of intracellular histamine (small vesicles, for example). The second is the observation of increased CD63 expression with concentrations of polyclonal anti-IgE that were supra-optimal for histamine release. This is difficult to reconcile with other studies and with the localization of the LAMPs to the granule membrane. As this was evaluated by single-color flow cytometry, and given the inconsistently bi-modal distribution of CD63 expression reported, perhaps the authors' own observations of platelet aggregation or some other contaminating cell are to blame (see below). This observation has been reported only with the use of polyclonal anti-IgE and, whatever the explanation, may not pertain to the use of CD63 in assays as discussed in this review. It does, however, deserve additional investigation, and suggests continued caution in equating phenotypic changes of basophils with the release of histamine.

Figure 1 CD63 up-regulation in response to allergen or anti-IgE activation



Ficoll-separated leukocytes from a peanut-allergic patient were placed in 96-well tissue culture plates in 100 μ l of complete serum-free medium. Then 100 μ l of medium containing 2 $\times$ IL-3 (4 ng/ml) and 2 $\times$ concentration of peanut extract, anti-IgE antibody or mock antibody was added to cells in triplicate. After 15 min, cells were harvested for analysis. Shown are results from one of three wells for each stimulant (a) and a summary of the results (b). PE, phycoerythrin; FITC, fluorescein isothiocyanate; CPE, crude peanut extract; PN, peanut.

Because CD63 is expressed broadly on leukocytes as well as on platelets, concerns have also been raised regarding the specificity of CD63 for the detection of degranulation rather than the adherence of basophils to

platelets – something that is known to occur with activated basophils [12]. These are important issues to consider, particularly for studies relying on few markers; however, numerous studies showing lack of CD41 (glycoprotein IIb/IIIa) expression and histology, including on the electron microscopy level, support the conclusion that this is not a routine problem.

Some of these concerns are more easily addressed due to the discovery of additional identification/activation markers. In 1999, Buhning *et al.* [13] identified a basophil/mast cell-specific marker using the monoclonal antibody 97A6, which they subsequently identified [14] as ectonucleotide pyrophosphatase/phosphodiesterase 3 (CD203c). The biological function of this ectoenzyme is not known, although it may play a role in basophil trafficking [15]; it has been described, however, as a marker of basophil activation [16,17]. In addition, activation is known to induce up-regulation of adhesion molecules such as $\beta 2$ integrins (CD11b, CD11c) [18] and very late antigen-4 (VLA-4) [19], and has been shown to result in secretion of an intracellular, basophil-specific epitope recognized by the monoclonal antibody BB1 [20]. The leukocyte early activation marker CD69 is also up-regulated by some basophil stimulants, particularly IL-3 [21]. Additional markers, such as IL-3 receptor α (CD123) [22] and the prostaglandin D₂ receptor (CRTH2) [23], have been used for gating on basophils as an alternative to IgE. Figure 1 shows an example of CD63 up-regulation on patient basophils induced by direct allergen or anti-IgE.

More recently, Hennersdorf *et al.* [24**] screened more than 260 monoclonal antibodies for reactivity with resting and activated CD203⁺ basophils. They identified four additional markers of basophil activation (CD13, CD107a, CD107b, and CD164) and, perhaps more importantly, helped to elucidate and expand upon what others had noted – that the CD63 and CD203c pathways of up-regulation are distinct [25]. This is important, as it may address what has been something of a controversy in the literature regarding which is the ‘best’ marker for measuring basophil activation [26–28]. What Hennersdorf *et al.* [24**] demonstrate nicely is that the LAMPs CD107a, CD107b and CD63 are up-regulated concomitantly, owing to their localization on granules. These are the same granules containing histamine, LTC₄ and other preformed mediators that are released upon Fc ϵ RI cross-linking and are distinct from the small vesicles associated with piecemeal degranulation. It is this second pathway that is suggested to be associated with up-regulation of CD203c, CD13 and CD164. Interestingly, two of these markers (CD13 and CD203c) are ectoenzymes.

These two pathways can be distinguished by kinetics, pharmacological inhibitors and differing sensitivities to

various stimulants. For example, both IL-3 and prostaglandin D₂ rapidly induce up-regulation of CD203c, but not CD63, and the phosphatidylinositol 3-kinase inhibitor, wortmannin, strongly blocks LAMP up-regulation, but not that of the CD203c group.

Often, CD63 up-regulation occurs in only a subset of activated basophils that up-regulate CD203c (W.G. Shreffler, unpublished observation, and [24**]), perhaps contributing to reports of increased sensitivity for the latter. One may need to define more carefully what is meant by activation, however. When the intended application is the diagnosis of immediate hypersensitivity, the use of activation markers not tightly coupled to degranulation is clearly problematic.

Given the reported capacity of Der p 1 and other allergens (as well as parasite antigens) to directly activate innate immune cells, including basophils [29–35], this response is of great interest; however, using this as a diagnostic test for immediate hypersensitivity may lead to more false positives. The group of CD203c markers may also be maximally up-regulated at baseline during periods of ongoing allergen exposure *in vivo*, limiting their use in direct basophil activation tests (BATs) (see below for discussion of systemic activation).

Any use of BATs must also contend with the issue of ‘non-releasers’; that is, individuals whose basophils are not responsive to Fc ϵ RI cross-linking. This phenomenon was described in early studies measuring histamine responses, and the prevalence has been estimated at ~10–20%. Several reports have identified defects in early signal transduction involving the tyrosine kinase Syk [36]. For at least some ‘non-releasers’, IL-3, which is known to prime degranulation responses, can reverse the defect *in vitro* after several days of exposure [19,37].

Use of basophil activation tests for diagnosis of food allergy

There have been several recent reviews on the use of BATs for the diagnosis of allergic disease [17,23,38]. I will focus in this review on the small body of literature specifically evaluating the use of BATs for food allergy.

An early application of a BAT for the diagnosis of food allergy was by Moneret-Vautrin and colleagues in 1999 [39]. They recruited 27 patients, characterized by serum IgE, prick skin test (PST), and provocation challenge, who were allergic to a total of 10 different foods. Seven of the patients were tested for two foods, resulting in a total of 34 tests. The authors performed LTC₄ release for all 34, and measurement of CD63 in 31 out of 34 tests. In addition, there were 24 control subjects on whom 35 activation tests were performed (all 35 for LTC₄ release, and 31 for CD63). When possible, if the subject’s

basophils were not responsive to IgE, then normal donor basophils were passively sensitized with the patient's plasma and these cells were stimulated with antigen. A positive response for LTC₄ was considered to be anything above 100 pg/ml; for CD63 up-regulation, a positive response was defined as > 5% CD63⁺ IgE⁺/total IgE⁺ above background. In this study, there were an extremely high number of anti-IgE non-releasers among both patients and controls (63% and 54% respectively), which the authors suggested may have been due to the anti-IgE antibody used or to the long delay (up to 24 h) between blood draw and assay. On combining direct and passive sensitization assays, however, they found 18 of 31 (58%) of patients to be positive by CD63, and 22/34 (65%) to be positive by LTC₄. All 18 CD63-positive tests were also positive by LTC₄. In the controls, 1/33 was positive by CD63, and 0/35 were positive by LTC₄ (specificity 97% and 100% respectively). Interestingly, 8/10 and 11/13 direct tests were positive, in comparison with roughly half when using passively sensitized basophils. The single false positive was also found in a test using passively sensitized cells, strongly suggesting that the direct test is superior, at least in terms of sensitivity.

In 2003, Erdmann *et al.* [40] published a comparison of the CD63-based BAT with specific IgE and PST for the diagnosis of pollen-associated food allergy. They recruited a total of 29 patients and 20 controls, and performed tests for specific IgE, PST (prick–prick and commercial extract), and BAT a total of 60 times for each group; on average, patients had evaluation for two tests, according to their history of allergy, while controls had evaluation for all three allergens (carrot, celery and hazelnut). Overall, BAT was positive 52/60 times against the relevant allergen, while 9/60 BATs were positive in controls. The false positives were individuals with detectable IgE by other tests as well.

The results of Erdmann *et al.* [40] were consistent with many other reports that the prick–prick method resulted in the highest sensitivity (100% for carrot and celery; 90% for hazelnut), followed by PST with commercial extract (sensitivities of 80%, 80% and 85% for carrot, celery and hazelnut respectively) and specific IgE (80%, 70% and 80% respectively). Specificities for all tests were between 80 and 90%. In comparison, BAT at the best cut-offs (determined by receiver–operator curve analysis for each antigen) was 85% sensitive for all three foods, with specificities of 80–85%. The sensitivities were strikingly similar to those with the PST using the commercial allergen extracts, which were the same as those used for the BAT. There is no comment on the issue of non-releasers by these authors. As they did not perform anti-IgE control stimulations, any patients with unresponsive basophils would simply be recorded as (potentially false) negatives.

The theoretical advantage of a biological test such as PST or BAT over specific IgE measurement for pollen-associated food allergy is in specificity. Since non-allergic individuals, rather than non-food-allergic individuals with pollen allergy, were used as controls by Erdmann *et al.* [40], the clinical usefulness of the study is severely compromised. The more pressing need for in-vitro testing for food allergy generally, and especially for pollen-associated food allergy, is for tests that discriminate cross-reactivity from true allergy [41]. In this study [40] the specificity was actually higher for the specific IgE testing. As the authors conclude, “These data suggest that the basophil activation test, like PST or allergen-specific IgE, does not provide definitive information with regard to the clinical relevance of sensitization.” [40].

A more recent study by Ebo and colleagues [42^{••}], however, does provide some of that evidence – at least in comparison with specific IgE. They addressed this issue directly, comparing 29 birch-allergic patients who had associated food allergy to apple with 22 birch-allergic patients without food allergy. They also included 10 healthy control subjects, and for all individuals they measured birch- and apple-specific IgE, performed PST to fresh apple and performed CD63-based BAT against apple extract. This design allowed the authors to compare sensitivity and specificity for the diagnosis of birch-associated apple allergy for each test in comparison with either normal controls or birch-allergic patients without associated apple allergy. Preliminary dose–response experiments demonstrated that basophils from birch-allergic patients would degranulate at high concentrations of apple extract [42^{••}]. Using an optimum allergen concentration (1 µg/ml) and receiver–operator curve analysis to define the best CD63 percentage cut-off (17%), sensitivity and specificity for food and birch allergy among birch-allergic individuals was 88% and 75% respectively. These were very similar to the values with PST (88% and 80% respectively) and compared favorably to the 30% specificity of specific IgE (sensitivity 96%). In comparisons with normal controls, sensitivity and specificity were both 100% (compared with sensitivities of 88% for PST and 96% for IgE). There were, however, three non-releasers (~10%), and if these are factored in as false negatives, the sensitivities fall to 80% for comparison with birch-allergic, non-oral allergy syndrome and 90% for comparison with normal controls. Even accepting this limitation, these are superior to the values obtained with measurement of specific IgE, and suggest that BATs may be an appropriate alternative or complement to PST.

Also in 2005, Erdmann *et al.* [43[•]] published an update to their use of the BAT for the diagnosis of pollen-associated food allergy – the first study to use recombinant food allergens for basophil provocation. They recruited a total

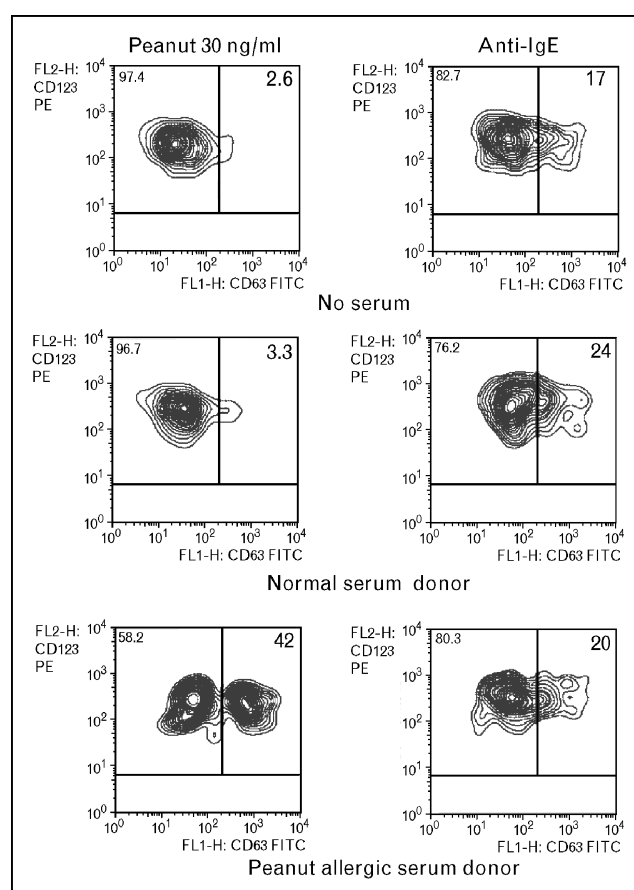
of 32 birch PST-positive patients, some of whom had multiple food allergies, into three groups of 20 each with clinical allergy (by history alone) to apple, carrot or celery. Ten normal controls with no evidence of sensitization by PST or specific IgE measurement were all negative by BAT. For calculating sensitivity and specificity, however, the authors appropriately compared results of BAT and other tests against clinical history within their atopic group. In other words, a patient with clinical allergy to apple only served as a negative control for carrot and celery. PST was performed by prick-prick with fresh fruit or vegetable and was 100% sensitive for all allergens, with specificities ranging from 64 to 91%. The sensitivity/specificity of allergen-specific IgE were 60%/64% for apple, 70%/86% for carrot and 75%/82% for celery. CD63-based BAT was performed using a single concentration of recombinant allergen for apple (Mal d 1), carrot (Dau c 1) or celery (Api g 1) that was determined by preliminary dose-response experiments with allergic individuals. Importantly, the authors did not report dose-response experiments with pollen-allergic, non-food-allergic controls. Given the findings of Ebo and colleagues [42**] discussed above, had they done so, they may have been able to determine a concentration that was maximally discriminating between the two groups. Nevertheless, given that single recombinant allergens were used, it is impressive that sensitivities were comparable with those of specific IgE testing. Recombinant allergen BAT was positive in 75% of apple- and carrot-allergic patients, while the sensitivity for celery allergy was 65%. Specificities were 68%, 77%, and 100% for apple, carrot and celery respectively. The unexpectedly high specificity of food allergen IgE testing in this patient population undermined any improvement in specificity from BAT (or PST).

Passive sensitization

Although not likely to become a routine diagnostic test, passively sensitized basophils have been used to evaluate functional characteristics of patient IgE. Two examples of this in the context of food allergy are cited.

This approach was used here at the Jaffe Food Allergy Institute at Mount Sinai to evaluate the functional differences of patient peanut-specific IgE. Using a peptide microarray-based immunoassay, we identified patients with highly diverse peanut allergen epitope recognition, and passively sensitized non-peanut-allergic donor cells without enrichment for basophils [44]. Basophil activation was then measured after provocation with peanut antigen, by up-regulation of CD63. Figure 2 demonstrates that passively sensitized cells acquired the capacity to degranulate in response to peanut antigen. This response correlated well with histamine measured in the supernatant ($r = 0.83$; $P < 0.001$; not shown). Consistent with the hypothesis that peanut-specific

Figure 2 Normal donor basophils can be passively sensitized to react specifically to peanut allergen

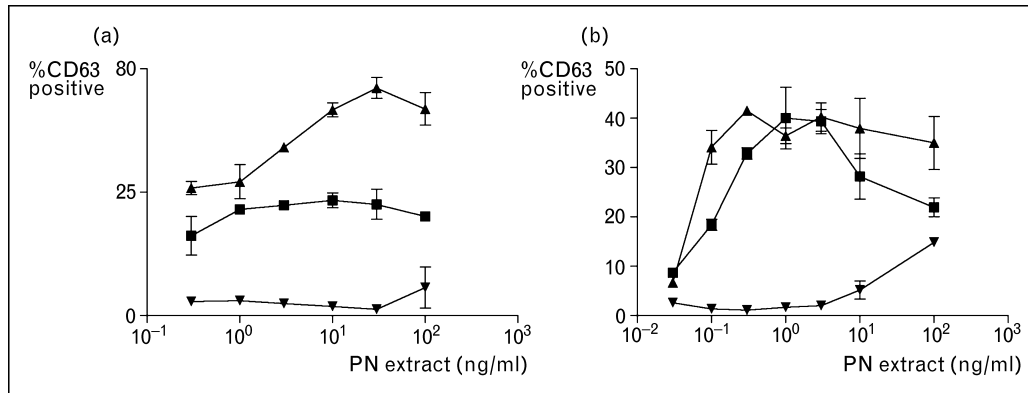


Ficoll-separated leukocytes were washed and placed in 96-well tissue culture plates with complete serum-free medium with or without 30% (v/v) patient serum for 1 h. Cells were then washed twice and stimulated described in the legend to Fig. 1 in triplicate. Only cells pre-incubated with serum from a peanut-allergic donor conferred sensitivity to peanut allergen. Responses to anti-IgE were present regardless of prior sensitization, as expected.

IgE recognizing many epitopes would have a greater capacity for triggering activation, both atopic and non-atopic donor cells degranulated more strongly when sensitized with IgE from patients with high epitope diversity (Fig. 3 and [44]). This was confirmed using the rat basophilic leukemia cell line SX38, which had been transfected with human FcεR [45].

The second example comes from de Leon and colleagues [46], who used passively sensitized leukocytes with affinity-purified peanut-specific patient antibody to test for cross-reactivity to tree nuts. They used sera from two individuals with peanut allergy and sensitization to several tree nuts to passively sensitize non-peanut/tree-nut-allergic donor cells. They concluded that peanut-specific antibody can be functionally cross-reactive with tree-nut allergens.

Figure 3 Serum with high sequential epitope recognition ('high diversity') confers greater peanut-specific responsiveness



Human Ficoll-separated leukocytes from a non-atopic (a) or atopic (b) non-peanut-allergic donors were sensitized with non-peanut-allergic sera (▼), 'low-diversity' peanut-allergic sera (■), or 'high-diversity' peanut-allergic sera (▲) and then stimulated with the indicated amounts of peanut (PN) allergen. Responses to peanut allergen were significantly greater when cells were sensitized with the high-diversity serum pool ($P < 0.05$ for atopic donor; $P < 0.001$ for non-atopic donor; two-way ANOVA). No difference was seen in responses to stimulation with anti-IgE (not shown).

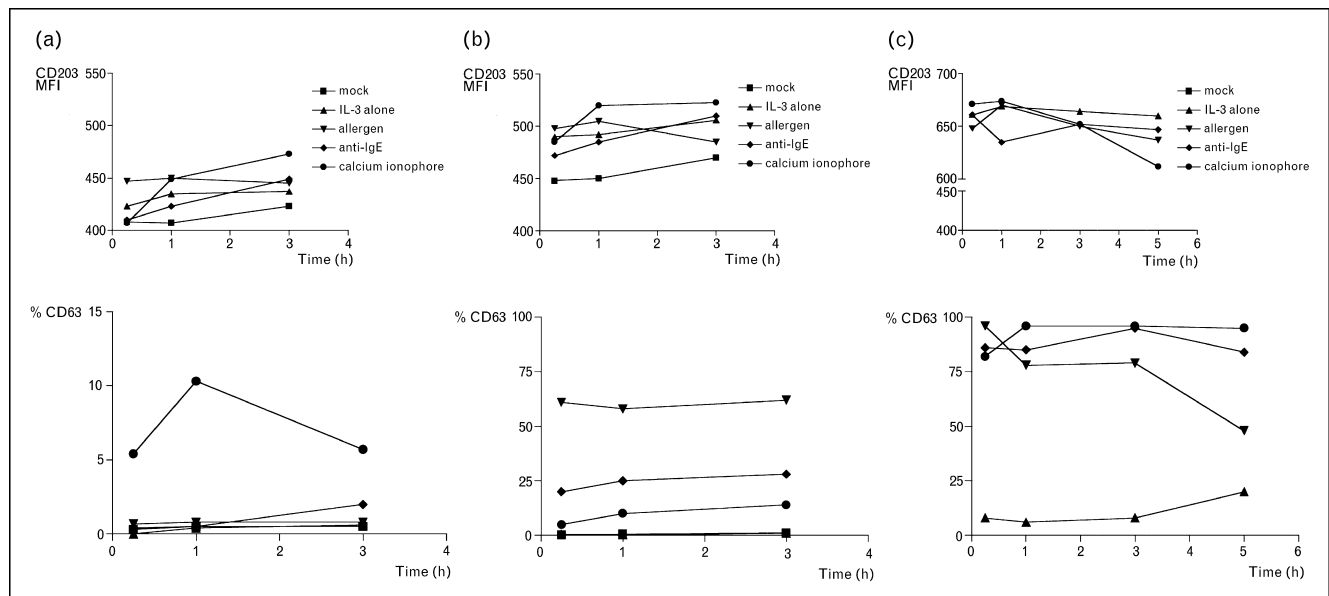
Systemic activation

Another potential use of monitoring basophil activation status may be for assessment of allergic inflammation/activation *in vivo* [47]. It has long been observed that a substantial percentage of food-allergic patients are found to have basophils that release histamine without *in-vitro* provocation [5,6,39]. Interestingly, these patients do not express high baseline levels of CD63 [39], although they may be more sensitive to degranulation due to specific and non-specific stimuli (W. Shreffler, unpublished

observation). This is consistent with so-called piecemeal degranulation, whereby granule contents are partially released by vesicle transport without extrusion of granules [48]. If this is the case, one would predict that the levels of vesicle-associated proteins, such as CD203c, CD13 and CD164 (see above), would be up-regulated at baseline.

We have, in fact, found that CD203c is expressed at very high levels in patients with atopic dermatitis and food

Figure 4 CD203c is an ex-vivo marker of basophil activation



Three donors were subjected to a direct basophil activation assay using whole blood and optimal doses of anti-IgE, allergen or calcium ionophore in the presence of 2 ng/ml human IL-3. Cells were stained with monoclonal antibodies to CD63, CD123 and HLA-DR (all from BD Biosciences) and monoclonal antibody 97A6 against CD203 (Immunotech). Basophils were gated as CD123⁺ HLA-DR⁺ cells, and were all CD203⁺. The percentage of positive CD63 (lower panels) and CD203c mean fluorescence intensity (MFI; upper panels) are shown at 0.25, 1 and 3 h. (a) Non-releaser who had no history of allergic disease and normal total IgE; (b) atopic dust-mite-allergic individual with no atopic dermatitis; (c) milk-allergic patient with atopic dermatitis and milk-containing diet.

allergy – the same patients reported previously as having high levels of spontaneous histamine production [5,6]. Figure 4 shows an example of the basophil phenotype of a patient like this. This patient's basophils also up-regulated CD69 in response to IL-3, as well as FcεRI cross-linking, more rapidly than those with lower baseline CD203c expression (not shown).

In fact, Yoshimura *et al.* [21] have suggested CD69 as a marker of basophil activation *in vivo*. Using quantitative flow cytometry, they found modestly higher baseline expression of CD69 on basophils from atopic donors than on those from non-atopic donors, but, more impressively, they found that basophils in bronchoalveolar lavage fluid expressed ~10-fold higher levels of CD69 than basophils from the peripheral blood of the same patients.

Lastly, the β-subunit of human FcεRI, which functions to amplify receptor signal transduction [49], has been shown to vary in its expression relative to that of FcεRIα by 10-fold in basophils from various donors, and IL-3 induces FcεRIβ expression [50]. Recently, the same group [51••] demonstrated that FcεRIβ protein and mRNA is up-regulated following in-vivo (nasal) challenge with allergen. They also found increased spontaneous IL-13 secretion from post-challenge basophil cultures, and this is consistent with the reportedly higher IL-3-induced IL-13 secretion from atopic compared with non-atopic donors [52]. The role of monomeric IgE, which is well known to regulate FcεRI expression [53], in the regulation of other aspects of basophil activation status that differentiate atopic and non-atopic individuals remains to be elucidated.

These studies together suggest that the measurement of basophil activation state, by surface marker, spontaneous cytokine secretion or expression of FcεRIβ, may have applications for monitoring in-vivo allergen exposure in our patients.

Conclusion

Although a tremendous amount has been learned about basophil activation, the assessment of basophil activation as a clinical tool for the diagnosis of food allergy is still in its infancy. It remains to be seen whether direct BATs will offer a diagnostic benefit, although they are likely to prove comparable to PSTs and may offer advantages such as greater standardization of testing and utilization for patients on anti-histamines. It is important to recognize that the activation and secretory pathways of basophils must be considered when choosing a surface marker-based BAT for diagnosis of immediate hypersensitivity. Monitoring the basophil activation state *ex vivo* for patients with food allergy on various elimination diets or following challenges with subjective or late reactions may also prove to be an important clinical tool, but much

work remains to apply this rapidly expanding knowledge of basic science to the bedside.

References and recommended reading

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

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 246).

- 1 Gibbs BF. Human basophils as effectors and immunomodulators of allergic inflammation and innate immunity. *Clin Exp Med* 2005; 5:43–49.
- 2 Devouassoux G, Foster B, Scott LM, *et al.* Frequency and characterization of antigen-specific IL-4- and IL-13-producing basophils and T cells in peripheral blood of healthy and asthmatic subjects. *J Allergy Clin Immunol* 1999; 104: 811–819.
- 3 Gauchat JF, Henchoz S, Mazzei G, *et al.* Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature* 1993; 365:340–343.
- 4 Saporta M, Kamei S, Persi L, *et al.* Basophil activation during pollen season in patients monosensitized to grass pollens. *Allergy* 2001; 56:442–445.
- 5 Sampson HA, Broadbent KR, Bernhisel-Broadbent J. Spontaneous release of histamine from basophils and histamine-releasing factor in patients with atopic dermatitis and food hypersensitivity. *N Engl J Med* 1989; 321: 228–232.
- 6 James JM, Kagey-Sobotka A, Sampson HA. Patients with severe atopic dermatitis have activated circulating basophils. *J Allergy Clin Immunol* 1993; 91:1155–1162.
- 7 Nakagawa T, Stadler BM, de Weck AL. Flow-cytometric analysis of human basophil degranulation. I. Quantification of human basophils and their degranulation by flow-cytometry. *Allergy* 1981; 36:39–47.
- 8 Metzelaar MJ, Wijngaard PL, Peters PJ, *et al.* CD63 antigen. A novel lysosomal membrane glycoprotein, cloned by a screening procedure for intracellular antigens in eukaryotic cells. *J Biol Chem* 1991; 266:3239–3245.
- 9 Azorsa DO, Hyman JA, Hildreth JE. CD63/Pltgp40: a platelet activation antigen identical to the stage-specific, melanoma-associated antigen ME491. *Blood* 1991; 78:280–284.
- 10 Knol EF, Mul FP, Jansen H, *et al.* Monitoring human basophil activation via CD63 monoclonal antibody 435. *J Allergy Clin Immunol* 1991; 88: 328–338.
- 11 MacGlashan DW Jr. Graded changes in the response of individual human basophils to stimulation: distributional behavior of events temporally coincident with degranulation. *J Leukoc Biol* 1995; 58:177–188.
- 12 Knauer KA, Kagey-Sobotka A, Adkinson NF Jr, Lichtenstein LM. Platelet augmentation of IgE-dependent histamine release from human basophils and mast cells. *Int Arch Allergy Appl Immunol* 1984; 74:29–35.
- 13 Buhring HJ, Simmons PJ, Pudney M, *et al.* The monoclonal antibody 97A6 defines a novel surface antigen expressed on human basophils and their multipotent and unipotent progenitors. *Blood* 1999; 94:2343–2356.
- 14 Buhring HJ, Seiffert M, Giesert C, *et al.* The basophil activation marker defined by antibody 97A6 is identical to the ectonucleotide pyrophosphatase/phosphodiesterase 3. *Blood* 2001; 97:3303–3305.
- 15 Salmi M, Jalkanen S. Cell-surface enzymes in control of leukocyte trafficking. *Nat Rev Immunol* 2005; 5:760–771.
- 16 Hauswirth AW, Natter S, Ghannadan M, *et al.* Recombinant allergens promote expression of CD203c on basophils in sensitized individuals. *J Allergy Clin Immunol* 2002; 110:102–109.
- 17 Buhring HJ, Streble A, Valent P. The basophil-specific ectoenzyme E-NPP3 (CD203c) as a marker for cell activation and allergy diagnosis. *Int Arch Allergy Immunol* 2004; 133:317–329.
- 18 Bochner BS, Sterbinsky SA, Briskin M, *et al.* Counter-receptors on human basophils for endothelial cell adhesion molecules. *J Immunol* 1996; 157: 844–850.
- 19 Kepley CL, Youssef L, Andrews RP, *et al.* Multiple defects in Fc epsilon RI signaling in Syk-deficient nonreleaser basophils and IL-3-induced recovery of Syk expression and secretion. *J Immunol* 2000; 165:5913–5920.
- 20 Kepley CL, Craig SS, Schwartz LB. Identification and partial characterization of a unique marker for human basophils. *J Immunol* 1995; 154:6548–6555.
- 21 Yoshimura C, Yamaguchi M, Ikura M, *et al.* Activation markers of human basophils: CD69 expression is strongly and preferentially induced by IL-3. *J Allergy Clin Immunol* 2002; 109:817–823.

- 22 Shreffler WG, Jarvinen K, Beyer K, Sampson HA. Characterization of basophil activation by three-color flow cytometry after passive sensitization with peanut allergic patient IgE: a simple bioassay without basophil enrichment for the evaluation of functional differences of patient peanut-specific IgE. *J Allergy Clin Immunol* 2003; 111:S470.
- 23 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.
- 24 Hennersdorf F, Florian S, Jakob A, *et al.* 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.
- These authors screened more than 260 monoclonal antibodies, identifying four recognizing three proteins that are up-regulated following FcεRI cross-linking. Using two-color flow cytometry, and measurement of LTC₄ and histamine as well as pharmacological inhibition, they conclude that these markers fall into two groups corresponding to different secretory pathways.
- 25 Monneret G, Boumiza R, Gravel S, *et al.* Effects of prostaglandin D(2) and 5-lipoxygenase products on the expression of CD203c and CD11b by basophils. *J Pharmacol Exp Ther* 2005; 312:627–634.
- 26 Boumiza R, Monneret G, Forissier MF, *et al.* Marked improvement of the basophil activation test by detecting CD203c instead of CD63. *Clin Exp Allergy* 2003; 33:259–265.
- 27 Ebo DG, Lechkar B, Schuerwegh AJ, *et al.* Comments regarding 'Marked improvement of the basophil activation test by detecting CD203c instead of CD63' by Boumiza *et al.* *Clin Exp Allergy* 2003; 33:849; author reply 852–843.
- 28 Hoffmann HJ, Bogebjerg M, Nielsen LP, Dahl R. Lysis with saponin improves detection of the response through CD203c and CD63 in the basophil activation test after crosslinking of the high affinity IgE receptor FcεpsilonRI. *Clin Mol Allergy* 2005; 3:10.
- 29 Haas H, Falcone FH, Schram G, *et al.* Dietary lectins can induce in vitro release of IL-4 and IL-13 from human basophils. *Eur J Immunol* 1999; 29: 918–927.
- 30 Ghaemmaghami AM, Robins A, Gough L, *et al.* Human T cell subset commitment determined by the intrinsic property of antigen: the proteolytic activity of the major mite allergen Der p 1 conditions T cells to produce more IL-4 and less IFN-γ. *Eur J Immunol* 2001; 31:1211–1216.
- 31 Phillips C, Coward WR, Pritchard DI, Hewitt CR. Basophils express a type 2 cytokine profile on exposure to proteases from helminths and house dust mites. *J Leukoc Biol* 2003; 73:165–171.
- 32 Svensson L, Rudin A, Wenneras C. Allergen extracts directly mobilize and activate human eosinophils. *Eur J Immunol* 2004; 34:1744–1751.
- 33 Traidl-Hoffmann C, Mariani V, Hochrein H, *et al.* Pollen-associated phyto-prostanoids inhibit dendritic cell interleukin-12 production and augment T helper type 2 cell polarization. *J Exp Med* 2005; 201:627–636.
- 34 Charbonnier AS, Hammad H, Gosset P, *et al.* Der p 1-pulsed myeloid and plasmacytoid dendritic cells from house dust mite-sensitized allergic patients dysregulate the T cell response. *J Leukoc Biol* 2003; 73:91–99.
- 35 Chen CL, Lee CT, Liu YC, *et al.* House dust mite *Dermatophagoides farinae* augments proinflammatory mediator productions and accessory function of alveolar macrophages: implications for allergic sensitization and inflammation. *J Immunol* 2003; 170:528–536.
- 36 Kopley CL, Youssef L, Andrews RP, *et al.* Syk deficiency in nonreleaser basophils. *J Allergy Clin Immunol* 1999; 104:279–284.
- 37 Yamaguchi M, Hirai K, Ohta K, *et al.* Nonreleasing basophils convert to releasing basophils by culturing with IL-3. *J Allergy Clin Immunol* 1996; 97: 1279–1287.
- 38 Ebo DG, Hagendorens MM, Bridts CH, *et al.* In vitro allergy diagnosis: should we follow the flow? *Clin Exp Allergy* 2004; 34:332–339.
- 39 Moneret-Vautrin DA, Sainte-Laudy J, Kanny G, Fremont S. Human basophil activation measured by CD63 expression and LTC₄ release in IgE-mediated food allergy. *Ann Allergy Asthma Immunol* 1999; 82:33–40.
- 40 Erdmann SM, Heussen N, Moll-Slodowy S, *et al.* 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.
- 41 Sicherer SH. Clinical implications of cross-reactive food allergens. *J Allergy Clin Immunol* 2001; 108:881–890.
- 42 Ebo DG, Hagendorens MM, Bridts CH, *et al.* 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.
- Perhaps the best study to date comparing standard PSTs and specific IgE tests against direct BATs using allergen extracts. Using controls without food allergy who were pollen-allergic, they found comparable sensitivity and superior specificity compared with specific IgE measurements.
- 43 Erdmann SM, Sachs B, Schmidt A, *et al.* 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.
- This paper was the first to use recombinant food allergens with a direct BAT. The sensitivity of individual recombinant allergens is quite good.
- 44 Shreffler WG, Beyer K, Chu TH, *et al.* Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. *J Allergy Clin Immunol* 2004; 113:776–782.
- 45 Dibern J, Donald A, Palmer GW, *et al.* RBL cells expressing human FcεRI are a sensitive tool for exploring functional IgE-allergen interactions: studies with sera from peanut-sensitive patients. *J Immunol Meth* 2003; 274:37–45.
- 46 de Leon MP, Drew AC, Glaspole IN, *et al.* Functional analysis of cross-reactive immunoglobulin E antibodies: peanut-specific immunoglobulin E sensitizes basophils to tree nut allergens. *Clin Exp Allergy* 2005; 35:1056–1064.
- 47 Bochner BS. Systemic activation of basophils and eosinophils: markers and consequences. *J Allergy Clin Immunol* 2000; 106:S292–S302.
- 48 Dvorak AM. A role for vesicles in human basophil secretion. *Cell Tissue Res* 1998; 293:1–22.
- 49 Lin S, Cicala C, Scharenberg AM, Kinet JP. The Fc(εpsilon)RIβ subunit functions as an amplifier of Fc(εpsilon)RIγ-mediated cell activation signals. *Cell* 1996; 85:985–995.
- 50 Saini SS, Richardson JJ, Wofsy C, *et al.* Expression and modulation of FcεpsilonRIα and FcεpsilonRIβ in human blood basophils. *J Allergy Clin Immunol* 2001; 107:832–841.
- 51 Saini S, Bloom DC, Bieneman A, *et al.* Systemic effects of allergen exposure on blood basophil IL-13 secretion and FcεpsilonRIβ. *J Allergy Clin Immunol* 2004; 114:768–774.
- A direct demonstration of the effects of in-vivo allergen exposure in a controlled setting on basophil activation status *ex vivo*.
- 52 Sin AZ, Roche EM, Togias A, *et al.* Nerve growth factor or IL-3 induces more IL-13 production from basophils of allergic subjects than from basophils of nonallergic subjects. *J Allergy Clin Immunol* 2001; 108:387–393.
- 53 Kawakami T, Galli SJ. Regulation of mast-cell and basophil function and survival by IgE. *Nat Rev Immunol* 2002; 2:773–786.