

CORRESPONDENCE

Regarding Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS & Stevens WJ. *In vitro* allergy diagnosis: should we follow the flow? Clin Exp Allergy 2004; 34:332–9

Comments regarding 'In vitro allergy diagnosis: should we follow the flow?' by Ebo et al. S. M. Erdmann, B. Sachs, K. Hoffmann-Sommergruber, O. Scheinerand H. Merk

We read the excellent review by Ebo et al. with great interest. Over the past years, we have been working on technical aspects of the CD63-based basophil activation test (BAT) and evaluating this novel method in different fields of allergy diagnosis such as allergy to foods, drugs, Hymenoptera and natural rubber latex applying receiver–operating curve analysis [1, 2]. We strongly believe that there is a role for this promising technique of *in vitro* diagnosis and would like to comment on some aspects addressed in this review.

To date mostly only natural allergen extracts have been applied to study allergen-induced CD63 expression. Hence, Ebo et al. suggest the benefit of applying recombinant allergens, for instance, as a basis for component-resolved allergy diagnosis should be evaluated in clinical studies. In a current study on birch pollen-associated allergy to foods we applied the recombinant allergens Bet v 1, Bet v 2, Api g 1, Dau c 1 and Mal d 1 in the CD63-based BAT using basophils from 30 food allergics with oral allergy syndrome induced by apple, carrot or celery and 20 controls. Results were compared with routine tests such as specific IgE (CAP method) and skin prick tests with native foods [3]. From this study, we draw several conclusions: (1) Application of recombinant allergens in the CD63-based BAT is feasible. (2) Sensitivity of the BAT for the three foods investigated ranged between 70% and 90%, exceeding sensitivity of specific IgE alone and increased overall sensitivity of *in vitro* tests. (3) In dose–response curves aiming at optimal allergen concentrations of the applied recombinant allergens a broad range of allergen concentrations (10^{-3} –100 ng) induced CD63 expression in allergics without resulting in reduced sensitivity. (4) The BAT did not differentiate between clinically relevant or irrelevant sensitization. (5.) In apple allergy in particular, use of Mal d 1 in the BAT considerably improved *in vitro* sensitivity as compared with specific IgE against apple (80% vs. 55%). This is of importance for practical use since application of a few or even one single allergen concentration will eventually considerably ease the use of the BAT in daily clinical practice. Further on, application of recombinant allergens in the BAT may contribute to elucidate patterns of cross-reactivity, for instance, in food or latex allergy. We are presently evaluating the BAT with a set of recombinant latex allergens (Hev b 2–8) in latex allergics (health-care workers, patients with Spina bifida, food allergics). Our data indicate that in the BAT, application of recombinant latex allergens is feasible as well, however with very distinct ranges of varying allergen concentrations for each recombinant allergen, and a pattern of sensitization characteristic of the different subgroups of

latex allergics with regard to source of sensitization. Interestingly, Ebo et al. demonstrated in a study on natural rubber latex allergy with natural allergen extracts that the BAT distinguished clinically relevant from irrelevant sensitization in several subjects [4].

Although we strongly support use of the BAT as a considerable progress of *in vitro* diagnostics we agree with Ebo et al. that quite a few open questions still remain and surprisingly to date several issues have not even been addressed at all. For instance, the problem of interference of prior drug intake such as glucocorticoids with the results of basophil activation as judged by CD63 expression or CD203c up-regulation has not been elucidated at all. In a small pilot study on the influence of oral administration of antihistamines we could not find a reduced rate of basophil activation in 10 birch pollen-sensitized patients after oral intake of 10 mg ceterizine as compared with basophil activation in the same subjects without intake of antihistamines (data unpublished). On the other hand with regard to some 'technical aspects' of this method (leucocyte suspensions vs. whole blood assays, pre-incubation with IL-3) some of these aspects might be of minor importance and in our eyes should not be overestimated. In another pilot study for technical evaluation of the CD63-based BAT, we demonstrated in 20 allergics (10 wasp and 10 birch pollen allergics) that the outcome of the BAT in an allergic subject (percentage of CD63⁺ basophils) was not influenced by the degree of basophil recovery within a broad range of 100–600 basophils. In other words, flow cytometric evaluation of a high quantity of basophils as compared with a low quantity does not improve the clinical and assay sensitivity, although at first sight this might be expected. With regard to the issue of pre-incubating basophils with IL-3 in a pilot study in 20 wasp allergics we established dose–response curves pre-incubating basophils with increasing amounts of IL-3 up to a final dose of 2 ng IL-3. Allergen-induced mean basophil activation was only increased by 5% by pre-incubating with IL-3 as compared with samples without pre-incubation with IL-3; however, priming with IL-3 may be helpful in drug allergy since drugs *per se* seem to induce a lower rate of basophil activation as compared with natural allergens in allergics [5].

The last point illustrates once more that at present not everyone should 'go with the flow' [6] but it is most desirable to establish multi-centre studies with uniform test protocols to tackle and answer these open questions and improve this promising *in vitro* method for daily clinical practice.

References

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Response by authors: *In vitro* allergy diagnosis: should we follow the flow? D. G. Ebo, M. M. Hagendorens, C. H. Bridts, A. J. Schuerwegh, L. S. De Clerck and W. J. Stevens

We have greatly appreciated the comments by Erdmann et al. about our review article [1], illustrating the potential of flow cytometric analysis and quantification of basophil activation as an *in vitro* instrument for allergy diagnosis and to assess the (residual) allergenicity of natural and recombinant allergens. In addition, the letter by Erdmann et al. creates the opportunity to briefly elaborate on some technical and clinical aspects of flow cytometry-assisted allergy diagnosis.

From their study in birch pollen-associated food allergy, the authors conclude that flow cytometric quantification of *in vitro* basophil activation did not discriminate between clinically relevant and irrelevant IgE. This finding is greatly in line with our own observations [2] but merits some comment. We believe that one of the most likely explanations why the basophil activation test better discriminates between clinical relevant and irrelevant latex IgE [3] as compared to clinical relevant and irrelevant IgE for pollen-associated food allergens has (to some extent) to be sought in the nature of the cross-reactive allergen. In birch pollen-associated food allergy the immunodominant cross-reactive structure between birch pollen and plant food is a protein. Both, clinical relevant and clinical irrelevant IgE antibodies to the foods implicated in birch pollen-associated food allergy generally result from sensitization to homologues of the major birch pollen allergen

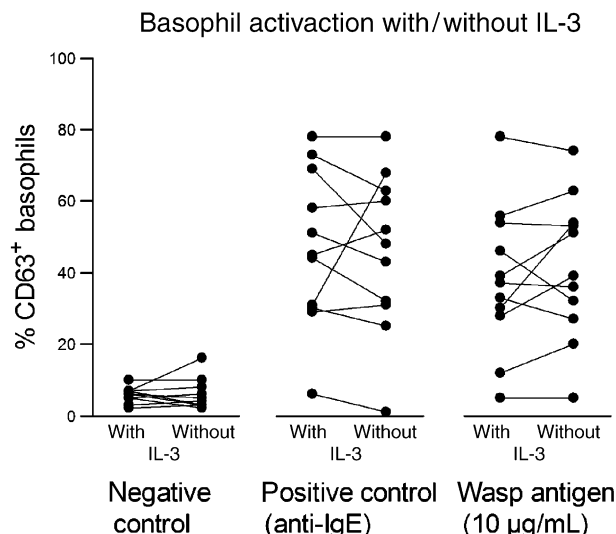


Fig. 1. Effect of pre-incubation with IL-3 (2 ng/mL). Pre-activated blood samples are stimulated with washing solution to measure spontaneous CD63 expression (negative control), anti-IgE (positive control) and relevant allergen (i.e. wasp venom).

(Bet v 1) [4]. Clinical irrelevant IgE antibodies to natural rubber latex, in contrast, generally results from sensitization against cross-reactive carbohydrate determinants (CCDs) from pollen proteins [4]. These pollen CCDs, on several occasions have been demonstrated to possess only a poor biological activity [5, 6].

On the other hand, although for a more definite interpretation of the data prospective investigations are needed, we think that some of the patients with a positive basophil activation test may reside in a sub-clinical transition phase between their birch pollen and secondary pollen-associated food allergy [7, 8].

We agree with the comment on pre-activation of the basophils with IL-3. At present, although comparative data are scarce, from the literature and our own experience it appears that pre-activation with IL-3 does not significantly alter the outcome of the basophil activation test. Figure 1, demonstrates that priming with IL-3 has no effect on wasp venom-induced basophilic CD63 up-regulation before venom-specific immunotherapy.

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