

CORRESPONDENCE

Regarding Boumiza R, Monneret G, Forrissier M-F et al. Marked improvement of the basophil activation test by detecting CD203c instead of CD63. Clin Exp Allergy 2003; 33:259–65.

Comments regarding 'Marked improvement of the basophil activation test by detecting CD203c instead of CD63' by Boumiza et al. D. G. Ebo, B. Lechkar, A. J. Schuerwegh, C. H. Bridts, L. S. De Clerck and W. J. Stevens

To our knowledge, in IgE-dependent diseases, a sensitivity as low as 50% and 75% has never been reported for flow cytometric analysis of *in vitro* activated basophils using the CD63 or CD203c antigen, respectively (review in [1]). For natural rubber latex, sensitivity of the technique readily exceeds 90% [2, 3]. Although the current observations might, to some extent, result from technical differences (e.g. IL-3 priming, unknown allergen stimulation concentration) one could argue whether the patients were correctly selected. From Table 1 it appears that five of these patients had a negative latex-specific IgE (sensitivity $\pm$ 69%), two of them even having a complete negative *in vitro* assessment. Currently, sensitivity of the Immuno-CAP is generally reported to vary between 75 and 90% (review in [4]). In this context providing the individual clinical data would certainly have improved the quality of the manuscript.

Second, and most importantly, flow cytometric results were considered positive when at least two sequential dilutions induced more than 10% CD63 or CD203c-positive basophils above control values. However, to our knowledge these threshold values have never been validated for natural rubber latex. In our opinion, and as recently addressed in two editorials in this Journal [5, 6], validation of novel diagnostic allergy tests absolutely needs establishment of allergen-specific threshold values (e.g. by ROC-analysis [7]). Threshold values should be calculated for CD203c and CD63.

Finally, in the absence of a χ^2 analysis, it remains an open question whether flow cytometric analysis of activated basophils is improved by using CD203c instead of CD63 for the diagnosis of natural rubber latex allergy.

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D. G. EBO, B. LECHKAR, A. J. SCHUERWEGH, C. H. BRIDTS, L. S. DE CLERCK and W. J. STEVENS
Department Immunology Allergy Rheumatology
University Antwerp, Universiteitsplein 1
2610 Antwerpen
Belgium
E-mail: immuno@uia.ua.ac.be

For allergy diagnostic flow cytometry, detection of CD203c instead of CD63 is not at all an improvement in other hands. A. L. de Weck and M. L. Sanz

In a recent publication in this Journal [1], the authors claim that the use of a monoclonal anti-CD 203c antibody instead of an anti-CD 63 antibody yields superior gating of basophils, a higher range of activation in response to allergen and represents a marked improvement of the basophil activation test.

Since this general conclusion does not correspond to our own experience and to some as yet unpublished results comparing anti-CD203c and anti-CD63 in our own protocol, we feel that some caution is warranted before recommending such switch. Boumiza et al. base their claim on the finding that among 16 patients with history of immediate-type allergy to latex and positive skin test, only eight (50%) were positive using a CD63 protocol while 12 (75%) were positive using an anti CD203c antibody. Furthermore, the intensity of basophil activation and the percentage of activated basophils were also markedly higher in their hands with the CD203c protocol than with the CD63 protocol (48% vs. below 20%).

About 18 months ago, following the publications by Platz et al. [2] on the properties of the new anti-CD203c antibody, we investigated the properties of that antibody (obtained from the same Immunotech source as used by Boumiza et al.) using our own CD63 protocol developed and optimized over several years [3]. We compared, using, not heparinized whole blood like Boumiza et al., but isolated buffy coat leucocytes, an anti-IgE-FITC, anti-CD63-PE protocol with an anti-IgE-FITC, anti-CD203c protocol in 10 patients with demonstrated IgE-mediated allergy to various allergens, including some to latex. Protocols and reagents were therefore quite similar to those reported by Boumiza et al. The results are shown in Table 1.

Table 1. Comparison of CD63 and CD203c protocols in 10 patients with IgE-mediated immediate type allergy to various allergens

Stimulus	Patient #1		Patient #2		Patient #3		Patient #4		Patient #5		Patient #6		Patient #7		Patient #8		Patient #9		Patient #10	
	63	203	63	303	63	203	63	203	63	203	63	203	63	303	63	203	63	203	63	203
Neg. control	8.2*	28	3.4	0.6	13.8	10.5	0.8	31.6	23.9	84.4	1.3	1.2	0	32.2	24.6	23.8	5.6	25.8	2.3	29
Pos. control†	80.6	19.3	89.7	87.3	93.7	7.8	65.9	74.4	86.5	80.3	83.5	18.0	88.7	94.0	98.2	34.5	78.0	35.6	95.4	50
Allergen dil 1	86.8	91.1	80.2	0.2	88.2	11.5	71.2	94.4	64.1	84.1	1.6	0.8	1.6	30.4	93.4	44.9	50.1	34.2	15.4	45.4
Allergen dil 2	82.1	86.8	71.9	3.9	90.6	7.2	68.3	76.3	76.4	71.3	0.4	0.1	0.7	15.4	96.5	77.5	18.7	23.1	4.4	36.4
Allergen	Peach		Peach		Parietaria		Parietaria		Peach		Latex		Latex		Peach		Parietaria		Peach	

Discrepant results are in bold. *, % basophil activation. †, pos. control is anti-IgE receptor. Allergens: Parietaria, dil 1/8, 1/32; Peach, dil 1/8, 1/32; Latex, dil 1/40, 1/160.

It is obvious from the Table that the results obtained with CD203c were generally inferior to those obtained with CD63 in terms of percentage of basophil activation than in terms of positive or negative patients. Furthermore, in contrast to the CD63 protocol in which negative controls may be clearly gated and distinguished from positive controls (anti-IgE receptor) and allergen-induced activation (Fig. 1), the CD203c protocol yields basophil clusters in which activated cells form a continuum with non-activated cells, making appropriate gating less obvious and the difference between negative and positive control less conspicuous. In several instances, the negative controls (non-stimulated basophils) with CD203c were above 10%, making the interpretation of the test difficult. Basophil recovery, on the other hand, was very similar for both protocols (Fig. 2). Accordingly, since our CD63 protocol yielded very good results (see below), further attempts to improve the 203c protocol were abandoned.

What may be the reasons for such discrepant findings? We believe that one reason may be the use of heparinized whole blood by Boumiza et al. and of isolated buffy coat leucocytes in our protocol. We have discussed elsewhere [4] the technical problems which may arise with whole blood, in particular a lower sensitivity to various allergens. Furthermore, CD203c is already present on resting basophils [2] and is detected merely as an increase upon activation while CD63 is hardly present on resting basophils. It may be easier to distinguish black from white than various shades of grey.

The motivation of Boumiza et al. to look for an alternative protocol to CD63 was the alleged disappointing sensitivity of CD63. Indeed, in their hands, only eight (50%) of patients with a history of IgE-mediated latex allergy and positive skin test demonstrated a positive CD63 basophil activation test while sensitivity increased to 75% with their 203c protocol. In both cases, specificity was excellent: 100%. We have recently published [3, 5] a comparable series of 43 latex allergy patients apparently very similar (positive history and skin test, specific IgE-positive was 88% in our study vs. 69% in the Boumiza study). In these 43 patients, the sensitivity of the basophil activation test, using our CD63 protocol [3] was 93% and the specificity 100%, with a positive predictive value of 100% and a negative predictive value of 99.9%. Furthermore, the average percentage of basophil activation was 58.9% in our series (range 17–92%) [5] while Boumiza et al. report a percentage of activation below 20%. Similar results have recently been published by Ebo et al. in 40 patients allergic to latex, finding a sensitivity of 93.1% and a specificity of 91.7% with a CD63 protocol [6]. It seems therefore indeed that the sensitivity reached by Boumiza et al. with their CD63 protocol is markedly lower than that achievable by others for a large number of protein allergens such as inhalants, foods, latex and insect venoms [3]. For such antigens, the sensitivity of our CD63 protocol was found near to optimal and various other modifications did not improve it.

For drugs, on the other hand, such as Betalactam antibiotics, metamizol or NSAIDs, the sensitivity of the CD63 protocol should still ideally be improved but our (limited) experience makes us doubt that this can be achieved with CD203c. For the time being and for drugs, a marked increase in sensitivity is obtained by combining the CD63 basophil activation tests with the ELISA determination of sulfidoleukotrienes (CAST) on the cells' supernatant [7].

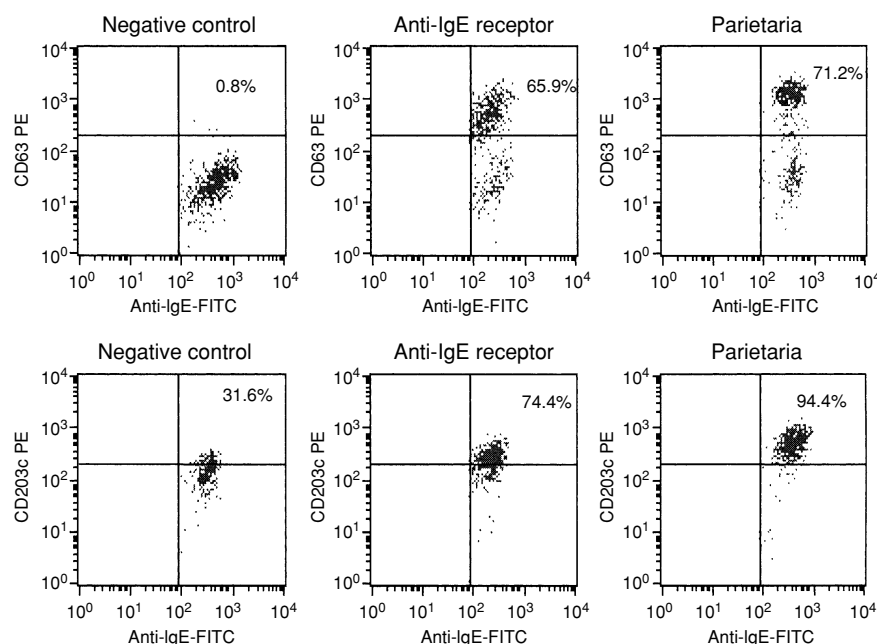


Fig. 1. Example of gating results using the CD63 (upper panels) or the CD203c (lower panels) protocols (patient #4). Notice that for negative control (left panels), positive control with anti-IgE receptor (middle panels), and allergen-induced stimulation (right panels), the distinction between activated and non-activated basophils is much easier with anti-CD63 antibody.

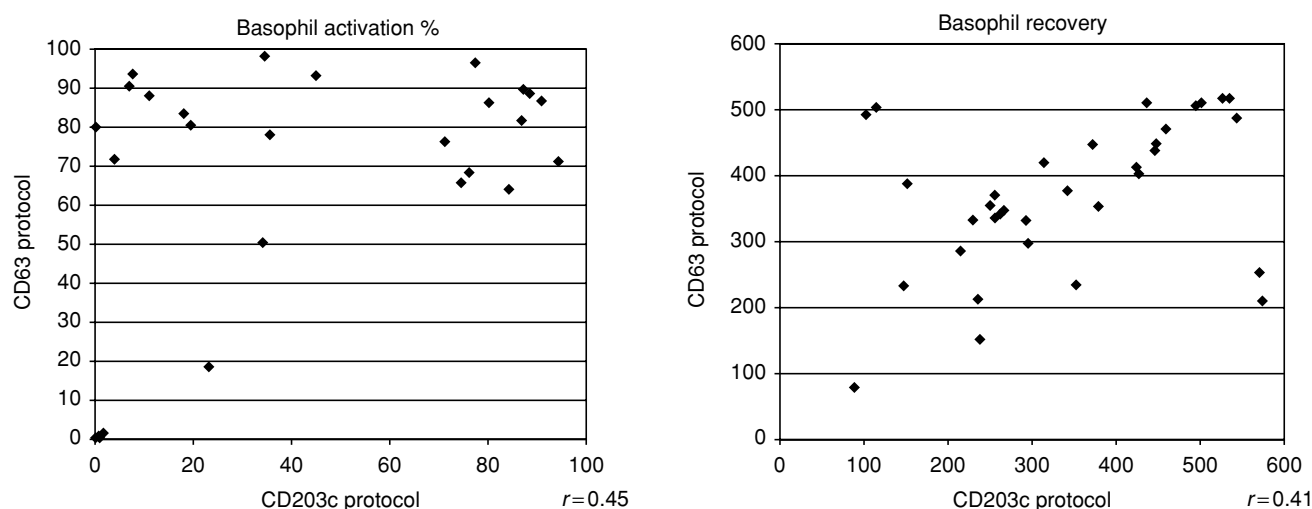


Fig. 2. Correlation between basophil activation induced by anti-IgE receptor and/or allergens (upper panel) and basophil recovery (lower panel) with an anti-IgE-FITC/CD 63-PE protocol or an anti-IgE-FITC/anti-CD 203c-PE protocol. Notice the relatively large number of activation percentage lower with the CD 203c protocol.

We believe therefore that advocacy of changes in established and clinically validated routine flow cytometric diagnostic protocols requires more extensive prior investigations and, as Boumiza et al. also suggest, multi-centric studies.

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A. L. DE WECK and M. L. SANZ
Department of Allergology
University of Navarra
Pamplona
Spain
E-mail: mlsanzlar@unav.es

Response by authors: Detection of CD203c is clearly an improvement for monitoring basophil activation in whole blood. G. Monneret, R. Boumiza and W. S. Powell

We have been among the first to develop the basophil activation test by flow cytometry and we have subsequently had extensive experience in monitoring CD63 expression in clinical studies [1–4]. In our hands, CD63 and IgE staining were unsatisfactory for clinical diagnosis due to low sensitivity. We believed that a major reason for such disappointing results might be that those two markers are not specific to basophils. Because CD203c is exclusively expressed on basophils, mast cells and their progenitors in blood, we compared its utility to that of CD63. Use of CD203c markedly improved the basophil activation test in our laboratory in terms of both clinical results (sensitivity) and flow cytometry (gating strategy, range of activation). In our study we compared basophil activation tests using either CD203c or CD63 (in exactly the same analytical conditions) in a well-characterized allergic patient group. The goal of our study was to improve the diagnosis of immediate-allergy in general using flow cytometry, and we only used latex allergy as an example. We found that for assessment of basophil activation CD203c is superior to CD63 and this constitutes, up to now, the sole published comparison of these two markers. It is difficult to evaluate the data reported by Sanz and de Weck because they were obtained in a small and heterogeneous group of allergic patients (allergies were due to three different allergens: peach, $n = 5$; latex, $n = 2$; parietaria, $n = 3$; which were tested in different conditions of allergen concentrations). Furthermore, after careful reading of the previous manuscripts by Sanz et al. [5,6], we identified several possible explanations for their results when comparing CD203c and CD63. First, there is a possibility that different CD63 results from laboratory to laboratory could be due to different degrees of labelling of CD63 with the fluorophore since we are using antibodies provided by different companies (Immunotech and Caltag). More importantly, the baseline values for CD203c expression in their negative controls (Table 1 and Fig. 1 in their comments) are much higher than those reported in both our paper and those of others focused on CD203c. This is likely to be due to the addition of IL-3 (2 ng/mL, 10^{-10} M) in their protocol for priming basophils. We found that this concentration of IL-3 induces a marked increase of CD203c (Fig. 3). Furthermore, Sanz and de Weck used isolated leucocytes which may result in cell activation whereas, we use whole blood without any priming mediators. The use of whole blood should reduce basal basophil activation

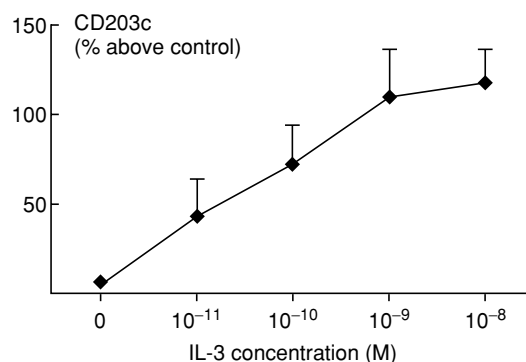


Fig. 3. Effect of IL-3 on CD203c expression by basophils from three healthy individuals. The results are expressed as the percentage of increase above control as mean $\pm$ SEM. CD203c was measured as previously described [7].

to a minimum, thereby increasing the sensitivity of the assay. We believe that the use of IL-3 should be reserved for secondary testing of non-responder patients.

Regarding the correspondence of Ebo and colleagues, we believe the authors misunderstood the main goal of our work. To explain our poor CD63 results, they suggest that our patients were not correctly selected although they all developed typical features of an anaphylactic reaction after contact with latex and allergy was then confirmed by positive skin testing. Even if we accept their argument, this would have no impact on our results because, once again, the goal of our study was not to improve the diagnosis specifically in latex allergy but rather to compare flow cytometric protocols for the diagnosis of immediate-type allergy in general. In our study the CD203c protocol identified 12 allergic patients whereas the CD63 protocol identified only eight of them. If we exclude, as proposed by Ebo et al., patients with negative latex-specific IgE, we still would find a better sensitivity for CD203c (72%) than for CD63 (46%). On the other hand, regarding thresholds values, we examined the use of different thresholds (5, 10 and 15%) as described in the results section of our study. Setting the threshold value is somewhat arbitrary, and for this reason we mentioned in our conclusion that 'the definition of a common gating strategy as well as the setting of the threshold value remains to be established for assessing this kind of test in multi-centre studies'. We found a threshold value of 10% to give the best results in our study in terms of specificity and sensitivity, but we did not wish to imply that this is the optimal values for basophil activation tests in general.

In conclusion, we agree that CD203c remains to be tested under similar conditions as ours before drawing any definitive conclusions. Basically, these two commentaries raise the question of standardized and proper use of flow cytometry for the diagnosis of allergy and we agree with the authors that multi-centre studies are mandatory to validate any marker before its clinical use, which is not yet the case for either CD63 or CD203c. We are sure that CD203c will not improve the diagnosis of latex allergy but we remain quite convinced that CD203c will improve the flow cytometric diagnosis of immediate-allergy.

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G. MONNERET*†, R. BOUMIZA* and W. S. POWELL‡

**Immunology Laboratory*

Lyon-Sud University Hospital

Lyon

France

‡Meakins-Christie Laboratories

Department of Medicine

McGill University

Montreal

Canada

†E-mail: guillaume.monneret@chu-lyon.fr