Letter to the Editor

Basophil activation test determination of CD63 combined with CD203c is not superior to CD203c alone in identifying allergic bronchopulmonary aspergillosis in cystic fibrosis

To the Editor:

We would like to comment on the letter from Chirumbolo[1] regarding the work of our collaborators Mirkovic et al.[2] Mirkovic et al demonstrated that *Aspergillus fumigatus*–sensitized individuals display an increased upregulation of the surface-expressed basophil marker CD203c in response to *ex vivo* challenge with *A fumigatus* extract, a response readily measurable by flow cytometry in the basophil activation test (BAT). However, Mirkovic et al did not report on evaluation of basophil CD63 expression, which is also upregulatd by allergen challenge, as shown in our previous published work.[3]

It is known that CD203c and CD63 have independent kinetics upon basophil activation. CD63 appears to be contained in the same cytosolic granules as histamine; CD63 surface upregulation and basophil histamine release thus have similar kinetics.[4] CD203c is an ectonucleotidase (E-NPP3) that is rapidly induced at the surface of activated basophils to hydrolyze ATP.[5] It has recently been shown that E-NPP3 is responsible for the suppression of ATP-dependent chronic allergic inflammation.[6] CD203c and CD63 responses in the BAT assay have been shown to be informative in identifying patients with insect venom allergy, food allergy, latex allergy, and major immediate-type drug allergies. However, in patients with cystic fibrosis (CF), Chirumbolo raised the important question as to whether measuring CD63 upregulation at the surface of the basophil in association with CD203c levels would be helpful in identifying allergic bronchopulmonary aspergillosis (ABPA) and/or discriminating patients with ABPA from those colonized with *A fumigatus* (without ABPA). To bring insight into this question, we reviewed our data on both markers.

We conducted a 2-year prospective longitudinal study comparing BAT biomarkers by flow cytometry before and after activation with *A fumigatus* allergen extract and recombinant *Asp f1* in patients with CF-ABPA (n = 20) and in 2 comparison groups: patients with CF colonized with *A fumigatus* but without ABPA (CF-AC; n = 13) and patients with CF without either *A fumigatus* colonization or ABPA (CF; n = 12). Patients were tested every 6 months and when ill with a pulmonary exacerbation. In this study, a number of basophil markers, including CD203c and CD63, were evaluated, although results for only CD203c were reported in our recent publication.[7] We here summarize our results evaluating the receiver operating characteristics (ROCs) of BAT CD203c responses alone or combined with CD63 (Table I).

In brief, the area under the ROC curve to identify patients with CF-ABPA as compared with patients with CF without ABPA or *A fumigatus* colonization, based on CD63 levels combined with CD203c, was only marginally superior to the ROC curve generated with CD203c alone at visit 2 (0.94 compared with 0.86) and the 2 ROC curves were identical at visit 1 (0.98). The ROC curve that resulted from CD203c combined with CD63 was similarly only slightly better than the ROC curve using CD203c only to discriminate patients with CF-ABPA from patients with CF with *A fumigatus* colonization (CF-AC) at visit 1 (0.90 compared

TABLE I. Predictive values of individual and combined blood basophil CD203c and CD63 levels after 10-minute *ex vivo* stimulation with *A fumigatus* extract for subject group

A. Visit 1

Groups to identify	Proposed predictor(s)	*P* value (log likelihood)	Area under the ROC curve
CF-ABPA vs CF	CD203c (10 min)	$<10^{-3}$	0.98
	CD63 (10 min)	.22	0.57
	CD203c × CD63 (10 min)	$<10^{-3}$	0.98
CF-ABPA vs CF-AC	CD203c (10 min)	.003	0.87
	CD63 (10 min)	.16	0.61
	CD203c × CD63 (10 min)	$<10^{-3}$	0.90
CF-AC vs CF	CD203c (10 min)	.12	0.60
	CD63 (10 min)	.91	0.52
	CD203c × CD63 (10 min)	.08	0.64

B. Visit 2

Groups to identify	Proposed predictor(s)	*P* value (log likelihood)	Area under the ROC curve
CF-ABPA vs CF	CD203c (10 min)	$<10^{-3}$	0.86
	CD63 (10 min)	$<10^{-3}$	0.85
	CD203c × CD63 (10 min)	$<10^{-3}$	0.94
CF-ABPA vs CF-AC	CD203c (10 min)	$<10^{-3}$	0.96
	CD63 (10 min)	.002	0.82
	CD203c × CD63 (10 min)	$<10^{-3}$	0.95
CF-AC vs CF	CD203c (10 min)	.03	0.73
	CD63 (10 min)	.48	0.51
	CD203c × CD63 (10 min)	.07	0.73

with 0.87) and both ROC curves were almost identical at visit 2 (0.96 compared with 0.95). Finally, the ROC curve that resulted from CD203c combined with CD63 was again only slightly better than the ROC curve using CD203c alone in discriminating patients with CF-AC from patients with CF at visit 1 (0.64 compared with 0.60) and the 2 ROC curves were identical at visit 2 (0.73). The same results were obtained after 30 minutes of *ex vivo* stimulation with *A fumigatus* extract (data not shown).

Thus, from this study, allergen-induced BAT CD203c surface upregulation appears to be a sufficient single biomarker for diagnostic purposes, and does not require additional measurement of CD63 to identify ABPA and/or *A fumigatus* colonization in patients with CF. Our data suggest that differences between a single CD203c BAT and a combined CD203c-CD63 BAT are neither highly significant nor stable over time, and therefore the added effort and expense needed to run a combination assay (along with the statistical handling of that data) are not justified. Further studies enrolling large numbers of patients will need to be conducted to definitively determine the clinical value of the CD203c and CD63 BAT assays. This is particularly the case of ABPA occurring with asthma, which remains to be investigated as aggressively as ABPA occurring in people with CF has been to date.

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