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Reply to Chirumbolo et al.

To the Editor,

The commentary of Chirumbolo et al.¹ on our recently published article² needs to be addressed in some issues that if not adjusted in this reply may lead to misunderstandings. The concerns give us the opportunity to provide the reader with more details on the clinical data of our patient and with deeper insights into our test performances that might have been too restricted in our original case presentation.

In their comment, Chirumbolo et al. refer to a possible association between whey proteins and caseins speculating on non-IgE-mediated allergic reactions to these denatured whey proteins associated with casein micelles leading to a type 2-mediated inflammation. In the mentioned reference from Mjösberg and Spits³, innate lymphoid cells are reported to produce IL-5 and IL-13 leading to dysregulation such as asthma and allergy. Chirumbolo et al. further supported their assumption of a non-IgE-mediated immune intolerance to milk proteins by pointing to a study of He et al.⁴

In fact, cow's milk allergy (CMA) is known to be a complex food allergy comprising a wide spectrum of manifestations from IgE-mediated and non-IgE-mediated CMA which makes the CMA diagnosis challenging. But in contrast to non-IgE-mediated allergy with mild-to-moderate or, more uncommon, more severe symptoms with delayed-onset (Venter et al.⁵), we report on a patient with an immediate-onset expression of IgE-mediated persistent cow's milk allergy existing since early childhood. The patient's clinical history clearly documents an acute onset of IgE-mediated reactions within minutes upon ingestion of already very small amounts ("traces") of cow's milk with the induction of severe cardiovascular symptoms. The anaphylactic score according to Müller was determined as III, and the clinical history and its IgE-based mechanism were confirmed by skin prick test in childhood and shortly before OIT (these data were not shown in the original publication) and positive IgE antibodies as shown in table 1 of our case report.² Chirumbolo et al. speculated on soy milk as a trigger for non-IgE-mediated food allergy. However, the patient's history lacks evidence for reactions to soy. Specific IgE against soy protein and single allergens rGly m 4, nGly m 5 and nGly m 6 were negative.

Again, in contrast to the concern of Chirumbolo et al. for not addressing the hypothesis of a non-IgE-mediated milk intolerance, the clinical data of a genuine cow's milk allergy were further supported on the molecular level by identifying different IgE-reactive caseins in cow's milk besides β -caseins, albumin and lactoferrin in human milk in immunoblot studies.

Also, Chirumbolo et al. pointed out that "... basophil responsiveness to polyclonal anti-IgEs (control) and milk allergens, should show a non-IgE-mediated response, rather than an IgE-mediated allergy": We suggest that Chirumbolo et al. refer to figure 2 of our publication.² The controls provided in figure 2A showed that the anti-IgE

control decreased from nearly 6% CD63 activation before omalizumab administration to 1% or less during anti-IgE treatment with omalizumab and immune modulatory therapy with OIT which is to be expected. In contrast, fMLP, used as a second control, activated the basophils by a non-immunological, non-IgE-mediated response, which did not decrease significantly under anti-IgE treatment. Next, Chirumbolo et al. remarked that after 6 months of anti-IgE therapy, reduced CD63 activation was stronger for breast milk than for cow's milk and for human rather than for bovine α -lactalbumin. To the best of our knowledge, we assumed that compared to human breast milk, bovine milk induced basophil activation at lower allergen concentrations, meaning that less bovine allergen is necessary for CD63 activation. This is in line with the EAACI Position Paper by Hoffmann et al.⁶ assessing basophil response reporting on the basophil sensitivity, defined as the allergen concentration, at which half of all reactive basophils respond.

Finally, we comment on the main criticism:

Chirumbolo et al. are very outspoken about their concerns regarding the threshold of the basophil activation test (BAT). This claim is based on the matter that the CD63 activation threshold is "usually" higher than 5%-10%, as referenced by Chirumbolo⁷, Chirumbolo⁸, Venter et al.⁵ who only take 3 markers into account or even use markers (eg CD193), for which the discussion, as to whether or not these are exclusive for basophils, is still controversial. We think we are sharing this dissatisfaction with Chirumbolo et al., who are discussing many pitfalls in BAT performances and gave the community many thoughts how to improve BAT in flow cytometry (Chirumbolo⁹, Chirumbolo¹⁰, Chirumbolo et al.¹¹). Taking this into account, and parallel to the paper using a CD123 phenotyping protocol to gate basophils within flow cytometry by Chirumbolo et al.¹¹, we performed a BAT in our laboratory with a high sensitivity and specificity as recently published by Schwager et al.¹² and referenced in our case presentation, using light scatter properties and 12 markers—instead of only 3—for a more precise identification of basophils resulting in a very low background of the PBS control (% CD63 expression: 0.69 ± 1.05 , $n = 30$, data not shown), in comparison with the routine BAT from Bühlmann with a given cut-off positive control anti-IgE >5%. For a detailed description of the gating strategy, we would like to refer to the paper by Schwager et al.¹² Additionally, we would like to mention that with our BAT, non-responders show no response through the IgE receptor when applying the positive control anti-IgE (see Figure 1 for non-responders tested in our test system [IDs 1344, 1336, 683]).

We hope to convince Chirumbolo and co-authors as well as the readers that the BAT in our hands has been improved to an extent that favorably affects both sensitivity and specificity.

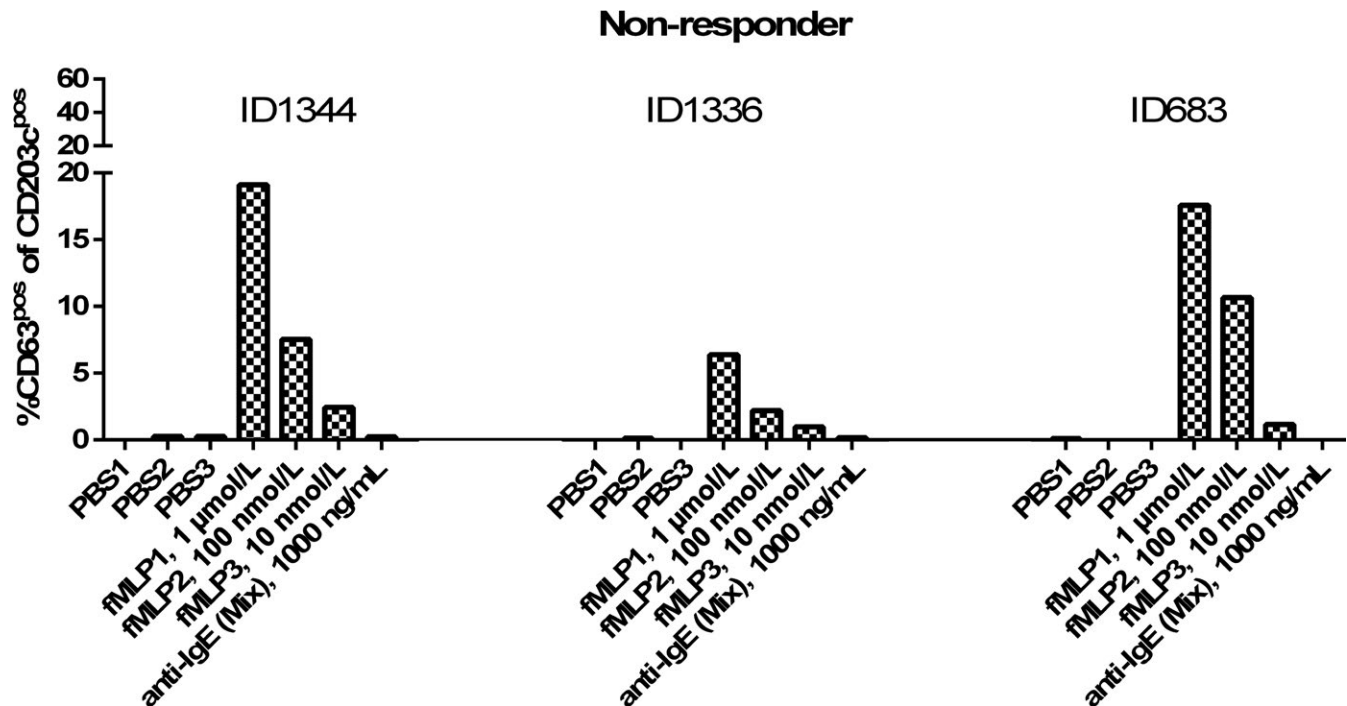


FIGURE 1 Results of basophil activation of three allergic patients who are regarded as non-responders

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