Whole blood high dimensional single cell functional profiling of basophils in peanut allergy

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September 4, 2022

Article Type : Letter to the Editor
Title : Whole blood high dimensional single cell functional profiling of basophils in peanut allergy

To the Editor:

Peanut allergy is the most common cause of anaphylaxis in childhood.¹ The acute IgE-mediated allergic reaction is initiated by basophils or mast cells via cross-linking of surface bound IgE through high affinity IgE receptors (FcεRI). Unlike mast cells, basophils circulate in the bloodstream and can be used to simulate the early events of allergen stimulation, with correlations to clinical outcomes.² Current approaches largely focus on peripheral blood derived mononuclear cells, which lack a significant percentage of basophils and limits interrogatory research on granulocytes. Less is known about the subtle molecular and cellular mechanisms that affect the innate immune system, specifically basophils, in peanut allergy and during oral immunotherapy (OIT). High dimensional immune profiling via mass cytometry (CyTOF) offers new possibilities to better understand innate cell populations in the context of food allergy. We aimed to a) develop a whole-blood stimulation mass cytometry approach to characterize basophil activation patterns and b) use this dataset to identify dynamics of basophil subsets, based on differences in expression of cellular markers and phosphorylation patterns upon allergen-specific stimulation. Such an approach could be applied to monitor desensitization during OIT at a high resolution.

We established a CyTOF panel to study differences in allergen-specific signaling pathways upon peanut stimulation in whole blood using barcoding. Fresh blood from clinically proven peanut-allergic (n=6) and non-allergic (n=3) children were obtained from The Hospital for Sick Children, Toronto, Canada. Samples were immediately stimulated with 0.01-100 ng/ml peanut extract for 3 or 15min. Phorbol 12-myristate 13-acetate-Ionomycin (PMA/Iono) and FcεRI-crosslinker (anti-FcεRI) were included as positive controls. Barcoded samples were pooled and stained with a panel of surface markers and anti-phosphorylation antibodies for p38, ERK, mTOR and Akt. Samples were acquired on a CyTOF Helios².

Relative basophil abundance did not differ between allergic and non-allergic individuals (Figure S1A). Moreover, there were no differences in expression of IgG receptors (CD16, CD32, CD64), IgE receptors (FcεRI, CD23) and other granulocyte markers (CCR3, CRTh2, IgE, CD123, Siglec8) (Figure S1B). The %CD63⁺ expression increased exclusively on basophils from peanut-allergic patients in response to peanut allergen stimulation, in a dose-dependent manner (Figure S2A-E).

Protein phosphorylation of Akt, MAP-kinases (ERK1/2, p38), and the mTOR pathway play crucial roles for the synthesis and secretion of cytokines,³ especially in the context of allergy.⁴ Phosphorylation of Akt, ERK1/2, p38 and mTOR were monitored at 3 and 15min after stimulation to measure basophil activation
downstream of the FcεRI receptor. Allergen-induced phosphorylation of ERK and p38 occurred in a dose-dependent manner at 3min, whereas phosphorylation of Akt and mTOR significantly increased at 15min (Figure 1A-B). Phosphorylation was significantly lower to absent in the non-allergic patients (Figure 1A-B). Phosphorylation status was subsequently assessed in both a successfully poly-desensitized pistachio-cashew-allergic (Figure S3A) and walnut-peanut-allergic (Figure S3B) OIT patient. From baseline, there was an initial increase in allergen-induced ERK phosphorylation in basophils at the end of OIT up-dosing. This change was followed by a partial return to baseline phosphorylation at the end of OIT, which may reflect the changes in basophil reactivity reported during OIT (Figure S3A-B).

To expand on existing knowledge in the basophil compartment, we developed a bioinformatic approach that allows changes to be monitored within the basophil subsets by complex normalization, pooling and clustering using the Leiden algorithm (Figure 2A-D). During stimulation, shifts in the proportion of stimulated basophils in subsets, as well as increased pERK and pmTOR marker expression (Figure 2E-F), were found and confirmed via gating.

In conclusion, we developed a whole-blood-based mass cytometry approach to assess basophil activation pathways that matched clinical reactivity and created an approach to investigate changes in basophil subsets during interventions for food allergy.

References


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Conflict of Interest Statement: XY, CHL, AC, CMD, JAH, AB, AK, and LH have nothing to disclose. JEMU reports grants/research support from DBV Technologies, Regeneron, CIHR, ALK-Abello, SickKids Food Allergy and Anaphylaxis Program, Advisory board for Pfizer, Kaleo, Bausch Health, Food Allergy Canada; in-kind drug donation from Novartis, other for Astra-Zeneca, all outside the current work. TE reports to act as local PI for company sponsored trials by DBV and sub-investigator for Regeneron, holds grants from Innovation Fund Denmark, CIHR outside the submitted work. He is Co-Investigator or scientific lead in three investigator-initiated oral immunotherapy trials supported by the Food Allergy and Anaphylaxis Program SickKids and serves as an associate editor for Allergy. He/his lab received unconditional/in-kind contributions from Macro Array Diagnostics and an unrestricted grant from ALK. He holds advisory board roles for ALK, Nutricia/Danone and Aimmune. TE reports lecture fees from Novartis.

Funding Information: This work was supported by The Hospital for Sick Children (Food Allergy and Anaphylaxis Program, start-up funds by the SickKids Research Institute and the Department of Pediatrics, Restracomp Graduate Scholarship to CHL, AK, and LH). CHL, AK, LH are the recipients of the Canadian Institutes of Health Research Fredrick Banting and Charles Best Canada Graduate Scholarship.

Statement of Author Contribution: XY, CHL, AC, CMD, JAH, and AB contributed to data acquisition. JEMU and TE contributed to experimental designs. XY, CHL, AC, AK, LH, JEMU, and TE contributed to data interpretation and preparation of the manuscript. The final version of the manuscript was approved by all authors.

Keywords: food allergy, pediatrics, mass cytometry

Abbreviations: CyTOF; cytometry by time-of-flight, OIT; oral immunotherapy, PMA/Iono; Phorbol 12-myristate 13-acetate-ionomycin, tSNE; t-Distributed Stochastic Neighbour Embedding

Word Count: 589/600

Figure Legends:

Figure 1. Πηγοπορφυλιτιον οφ πΕΡΚ 1/2 (πΤ202/πΨ204), π38 (πΤ180/πΨ182), Ακτ (σ473) ανδ μΤΟΡ ιν βασσικες οφ αλλεργις (ν=6) ανδ νον-αλλεργις ινδιvιδυας (ν=3) υπον στιμυλατιον ωιτη πεανυτ αλλεργεν, ΠΜΑ/Iono. Ορ αν αντι-ΦςεΡΙ αναι σενενε δια μας ψυμοτερψ.(A) A histogram illustration of the median changes in protein phosphorylation in basophils of allergic (n=6) and non-allergic patients (n=3). (B) Line graph illustrating median changes in protein phosphorylation in basophils of allergic (n=6) and non-allergic patients (n=3) after 3 or 15min post-stimulation with peanut extract (0.01-100 ng/ml) expressed as mean ± SEM.

Figure 2. Basophil subset clustering by stimulation and marker expression. (A) Basophils pooled from peanut-allergic patients (n=10) clustered with Leiden algorithm. (B) Basophil proportions stimulated by PMA/Iono, anti-FcεRI, peanut extract, and unstimulated in subsets 0-6. (C) Clustered basophils from peanut-allergic patients stimulated with PMA/Iono, anti-FcεRI, 10 ng/ml peanut extract, and unstimulated. Cells separated by duration of stimulation (0, 3, 15min). (D) Proportion of 3 and 15min stimulated basophils per subset. (E) Pseudo-time lineage plot of basophils from peanut-allergic individuals. Basophils transition from subsets 0 and 1 to subsets 2 and 4. (F) Subset expression of pERK, mTOR and p38.
(A)

(B)

(C)

(D)

(E)

(F)