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Background

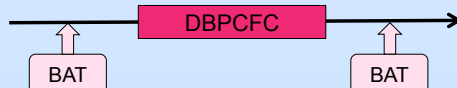
- The mechanisms which result in acute symptoms during IgE-mediated food allergic reactions are not clear, in particular:
 - Why do some food-allergic individuals tolerate relatively large amounts of allergen and only experience mild symptoms, while others develop anaphylaxis to 1/70 of a peanut?
 - How does orogastric exposure to allergen result in rapid-onset skin symptoms, in the absence of other systemic symptoms?
- We hypothesized that systemic basophil activation is a crucial step through which ingestion of food allergen leads to systemic effects.

Objectives

- To assess systemic basophil activation during *in vivo* exposure to peanut in peanut-allergic individuals
- To determine how this varies, depending on the presence of significant systemic symptoms.

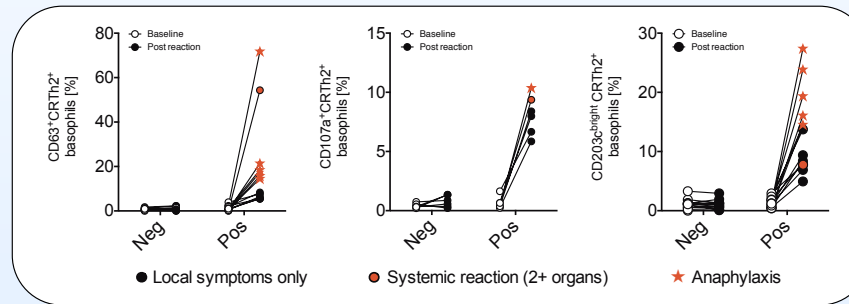
Methods

- Peanut-allergic individuals were recruited for this pilot study and underwent double-blind, placebo-controlled food challenge (DBPCFC) to peanut.
- DBPCFC were performed according to the PRACTALL consensus, modified slightly to include only objective symptoms as stopping criteria
- Whole blood was collected before and at the termination of DBPCFC.
- Activated basophils were quantified by flow cytometry, through measurement of expression of CD63, CD203c^{bright} and CD107a on CRTh2⁺CD303⁺CD123⁺ basophils following:
 - In vivo* exposure to peanut/placebo during DBPCFC, without *ex vivo* peanut stimulation
 - Ex vivo* incubation with increasing doses of peanut (0, 1, 3, 10, 33, 100, 330 and 1000 ng/mL), in blood collected both prior to and at termination of DBPCFC to peanut.
- Blinding of clinical & laboratory staff was maintained throughout until analyses had been completed.



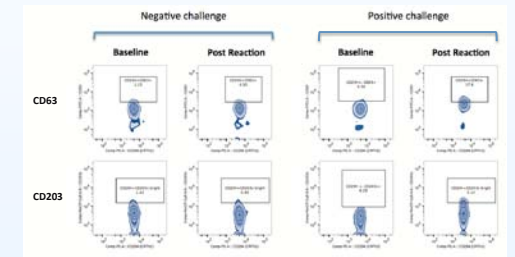
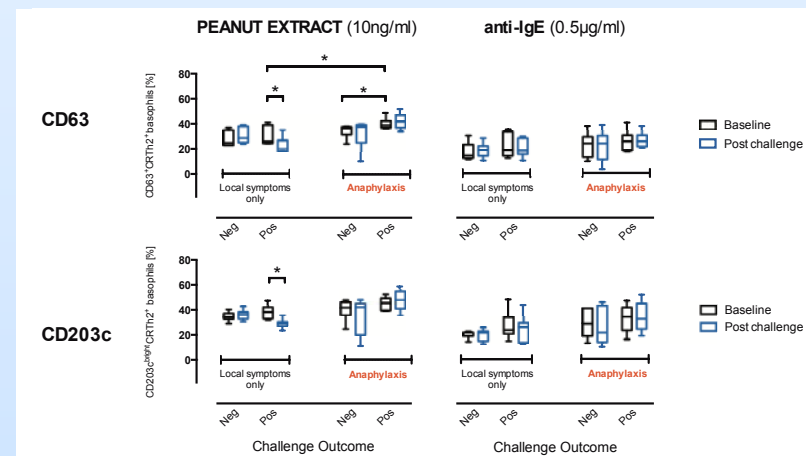
Results:

- Thirteen peanut-allergic individuals underwent DBPCFC: 7 experienced significant systemic reactions affecting 2+ organ systems, 6 of which were treated with epinephrine.
- Proportions of CD63⁺, CD107a⁺ and CD203c^{bright} CRTh2⁺CD303⁺CD123⁺ basophils were increased following positive (Pos) peanut challenge in human subjects (P < 0.01).**
- Expression of activation markers on basophils was greater in subjects with anaphylaxis during DBPCFC and given epinephrine, compared to those experiencing local symptoms (P < 0.05)



Subjects experiencing anaphylaxis at DBPCFC had:

- Increased expression of CD63 on basophils following *in vitro* exposure to peanut extract at baseline (prior to challenge).
- Persistent expression of CD63 and CD203c following further *in vitro* stimulation with peanut, in contrast to those with localized symptoms in whom basophil responses to further peanut exposure were diminished post challenge (P < 0.05)



ABOVE: Representative FACS plot

LEFT: Expression of CD63⁺, CD107a⁺ and CD203c^{bright} on CRTh2⁺CD303⁺CD123⁺ basophils prior to, and following positive (Pos) and negative (Neg) DBPCFC to peanut

Conclusions

- Our data imply an important role for basophils in the evolution of acute IgE-mediated reactions to food allergens.
- The degree of basophil activation may be associated with symptom severity.
- Anaphylaxis may be a consequence of impaired switch-off mechanisms in basophils following allergen exposure.**
- The involvement of basophils during acute allergic reactions to food may explain the high frequency of systemic symptoms in allergic reactions triggered by food, in contrast to other atopic conditions e.g. allergic airways disease.
- Further work is ongoing to identify the key events, which trigger widespread basophil activation in peanut allergy.

Acknowledgments

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