Reviewer's report

Title: Rhinosinusitis derived Staphylococcal enterotoxin B plays a possible role in the pathogenesis of food allergy

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Reviewer: Harumi Jyonouchi

Reviewer's report:

General
This paper addresses important clinical questions prospectively whether sinus inflammation in association with Staphylococcal colonization aggravates food allergy. It appears that their hypothesis is that SBC transmitted through post-nasal drip significantly augment food allergen-specific Th2 responses and allergen-specific IgE production. Improvement of clinical features and PST reactivity in the CRS patients with FA following sinus surgery is very interesting. However, I have problems of the study design (mainly defining study subjects) and results of assays measured as described below.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

• Introduction
2nd paragraph: The better rationale for hypothesis is required. It seems that authors first tried studies in animal models and on the basis of findings in the animal study, this prospective clinical study is designed.

• Methods
Subjects: It is essential to describe the study subjects more precisely. Usually in most allergy journals, the first table details demographic data of the study subjects including age, sex, allergy history (AD, AR, asthma, AC), prick skin test reactivity to food allergens and aeroallergens, total IgE levels, natures of CRS (hyperplastic sinusitis with polyposis, hypoplastic?, treatments, etc.), etc. Normal control are all young medical students and not age-matched to the CRS study subjects. It is important to state how many control healthy and CRS patients revealed atopic predisposition (absence of food allergy does not exclude presence of other atopic condition.)

• Results
Page 16, Fig. 3 and Table 4: The results of frequency of IFN-? and IL-4 expressing cells indicates that SEB per se increased the frequency of IL-4+ T cells without food antigen. The frequency of IL-4+ cells with SEB and Ag appears to be additive with frequency observed with SEB alone and with food Ag alone. If SEB serves as adjuvant just augmenting specific Ag responses, significant increase of IL-4+ cells with SBE alone is unlikely to occur. If SEB acts as a polyclonal stimulant skewed to Th2 responses, increase of IL-4+ T cells should occur in both CRS with FA patients and FA alone patients. In that case, SEB should also increase IFN+ T cells in CRS patients. The responses observed in the results appears that CRS with FA patients appear to be sensitized to SEB due to high colonization of S. aureus and revealing T cells responses to SEB. While other control groups lack SEB specific T cell responses. Control CRS patients and healthy volunteers appear to have very high background of IFN-g. It is unusual to see such high IFN-g production without stimulants by PBMCs. In our experience, IFN-? production is <3.7 pg/ml without stimulants if obtained from healthy controls.

Page 16, Fig 4. Likewise, Response to SBE may be just reflecting stimulation of SBE specific Th2 cells and resultant release of IL-4.
Discussion
I am not convinced with interpretation of results and hence, I do not agree with a major part of discussions. Almost all the results indicates that in CRS with FA patients, SEB in post-nasal drip are aggravating Th2 responses to food allergens in these patients by provoking SEB-specific Th2 responses in the gut mucosa (bystander effects?). CRS without FA patients appears to have Th1 skewed immune responses and even they have staphylococcal colonization, their responses may not be augmenting IgE mediated food allergies. Also judging from SEB concentration data in sinus lavage fluid, control CRS patients appears to have less SEB concentration than CRS with FA patients, perhaps reflecting less degree of Staphylococcal colonization in control CRS patients than CRS with FA patients? It is of note that SEB specific IgE levels are elevated in atopic dermatitis patients colonized with SEB producing Staphs.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

- Abstract
  o Background; the first two sentences should be deleted (duplicating in introduction).
  Rewriting is desirable for better understanding such as ‘SEB is a potent immunomodulator and implicated with pathogenesis of inflammatory diseases mediated by Th1 or Th2 dominant immune responses.’
  o Methods
    Rewriting for better understanding is required. Example; We have assessed changes in allergen skin test reactivity, serum levels of allergen specific IgE, IL-4, IL-13, IFN-?, and reactivity of PBMCs against food allergens and SBE following sinus surgery in CRS patients with or without food allergy. In CRS patients with FA, their responses to oral challenge of causative food allergens were also assessed before and after sinus surgery.
  o Results
    Not clearly described. I am not certain if I can completely agree with their interpretations (See Major Compulsory Revision section)
  o Conclusion
    Better to rewrite to reflect the results a little more accurately.

- Introduction – better to rewrite in general.
  Page 3, 1st paragraph; life threaten ?life-threatening
  Page 3, 2nd paragraph; I think that the authors want to state that "IgE mediated food allergy is believed to be mediated by type 2 T (Th2) cell responses to food allergens. In response to food allergens, Th2 cell produce Th2 cytokines including IL-4, IL-5, and IL-13. IL-4 and IL-13 promote food allergen-specific IgE production, leading to food allergen-induced mast cell activation." Description of memory T cell portion should be shortened, since the study data do not directly address the fate of memory T cells.
  Page 4, 1st paragraph: Nasal polyposis is not necessarily Th2 mediated.
  Page 4, 2nd paragraph: these should be integrated in Abstracts and Result section. Usually Introduction section should state hypothesis clearly and briefly describe what was addressed in the study.

- Methods - In general, it needs to be rewritten for better understanding.
  Page 7, prick skin test – it is necessary to rewrite the last sentence. The meaning is unclear.
  Page 7, oral challenge test; I assume that the study subjects underwent DBPC oral challenge test if they revealed positive prick skin test reactivity to food allergens. Please clarify.
  Page 9, SBE measurement paragraph: this part can be shortened substantially.
Page 11, Antigen specific CD4+ cell proliferation assay; the description is incorrect. The authors measured cytoplasmic expression of cytokines (IFN-γ and IL-4) by PBMCs in response to SBE and specific food allergen to which the study subject revealed the most significant reactivity in ST. They also tested IL-4 production by PBMCs. They are not technically considered as CD4+ cell proliferation assays.

• Results; Each paragraph tends to contain sentences associated with methods and such redundancy should be eliminated.
Page 13, the first paragraph ? should be integrated into the method section as description of the study subjects except for changes in sinusitis symptom scores.
Page 14, serum cytokine levels and Fig. 1; In general, it is difficult to detect 200-600 pg/ml levels of IL-4 or IL-13 in the serum. It almost looks like the results of cultured cells with stimulants. Likewise, IFN-g serum levels appear too high even in controls.
Page 15, Staphylococcus aureus cultures; it is necessary to state how many CRS patients without FA revealed positive Staphylococcal cultures and degree of colonization.
Page 16, flow cytometric results; it needs to be clarified that what authors checked is frequency of IL-4 and IFN-g expressing cells in response to SBE or food allergens.

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.