Author's response to reviews

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

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Author's response to reviews:

Dear Editors:

We have revised the paper according to the reviewer's comments. The answers are typed right below each question. Please note, page order has been changed a little because of the revision.

Thank you

Pingchang Yang

Reviewer's report

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

Harumi Jyonouchi Reviewer:

Reviewer's report:

Comments:

Introduction (Background)
Page 3, the first paragraph:
Probably 2nd sentence is unnecessary.
Answer: Yes. We have deleted the sentence.

3rd sentence: abdominal inconvenience A you mean abdominal discomfort?
Answer: Yes. We have replaced the word with "discomfort".

4th sentence: I assume that the authors meant?
Avoidance of offending food is the only effective therapeutic measure currently.
Answer: Yes. We have replaced the sentence with this one.

5th sentence: I think that the authors meant;
FA are prevalent irrespective with sex, ethnicity, age, and social/economic class and imposes a heavy burden on health care expenditure and global economy.
Answer: Yes. We have replaced the sentence with this one.

Page 3, the 2nd paragraph:
The first sentence: may consider revise the sentence such as written below:
Although etiology of FA is still not well understood, significant progress has been made for the recent few years with regard to molecular mechanisms of FA.
Answer: Yes. We have replaced the sentence with this one.

3rd, 4th, 5th sentences: not clearly written (you need to discuss in relation with FA). Please revise.
Answer: Yes. We have revised these sentences.

Page 4, the 1st paragraph:
Please correct several typo- or grammatical errors.
Answer: Yes. We have done.

3rd sentence; probably unnecessary.
Answer: Yes. We have deleted this sentence.

It seems that the authors want to state:
Antigen-independent, polyclonal T cell activation by SEB can worsen inflammatory condition in both Th1 mediated and Th2 mediated diseases including chronic rhinosinusitis. Thus controlling staphylococcal colonization or infection may have therapeutic implications in these conditions. It is reported that prophylactic antibiosis for Staphylococcus aureus revealed protective effects in atopic dermatitis. However, a role of superantigens in FA is not well understood.
Answer: Thanks. This is what we want to demonstrated. We have replaced the sentences with these ones.

Page 4, the 2nd paragraph:
2nd sentence: please clarify better.
Answer: Yes. We have revised this sentence.

3rd and 4th sentences: The authors meant as follows?
Our previous studies in rodents revealed that concurrent oral administration of SEB and food antigens (by gavage feeding?) induces IgE-mediated reactivity to food antigens in intestinal mucosa.
Answer: Yes. This is what we want to say. We have replaced the sentence with this one.

Hypothesis needs to be stated first and then explain how the prospective study was designed using what kind of outcome measures. The last sentences are explanation of obtained results and should be deleted or very simplified such as The obtained results in this prospective study did support a role of SEB in development or aggravation of FA in CRS patients.
Answer: We have revised this paragraph accordingly.

Methods overall please consider further editing in terms of English writing.
Answer: Yes. A native colleague has revised English for this paper.

Page 6
Study Subjects A it is hard to believe that all the CRS-FA patients are not sensitized with common aeroallergens like dust mite. Secondary to persistent CRS symptoms, a diagnosis of allergic rhinitis may be difficult without prick skin testing. Also atopic or non-atopic asthma is a common complication in CRS patients. No asthma diagnosed in the all the CRS study subjects? Skin prick test A Prick skin test? Extract of each fresh food A extract of each unprocessed food?
Answer: For the purpose of focusing on food allergy, we excluded those with other allergic diseases. We tested the responses to some common food antigens that does not deny a possibility that the patients also sensitize to other allergens. The food antigens we used in this study were bought from the Antigen Laboratory at Beijing Xiehe Hospital (a commercial antigen extract producer).

Page 7
Oral challenge test
One of the positive antigens from the skin test A one of the food allergens that the study subject revealed positive prick skin test reactivity?
Answer: Yes. We have revised this sentence.

10 common food allergens A it sounds like that these are used in prick skin testing.
Answer: Yes. We have revised this sentence.

The symptoms of food allergy attack are closely observed --: A The study subjects were closely monitored for 4 hours following oral challenge in the facility where treatment of anaphylaxis was readily available.
Answer: Yes. We agree. The sentence has been replaced with this one.

Cytokine and antigen-specific IgE assessment: Serum levels of cytokine and allergen-specific IgE levels?
Answer: We have revised this sentence.

Some sentences are unclear and editing is recommended.
Answer: Yes. We have done some.
Antigen-specific T cell proliferation assay: (CD4+ cells are not purified)
Answer: No. We did not purify CD4 T cells. If we did, extra antigen presentation cells would be required to carry out the antigen presentation. Since there were antigen presentation in LPMCs, that made the experiments executable. We described CD4 T cell in this paragraph indirectly based on the factor that we measured cytokine levels of Th1 and Th2 in the culture; we also counted the cell population of Th1 and Th2 with flow cytometry.

1st paragraph;
an antigen with the largest wheal area in the skin prick test is an food allergen to which the study subject revealed the most significant prick skin test reactivity?
Answer: Yes. We agree. This sentence has been revised.

For intracellular cytokine staining, how many hours are cells incubated? (Usually 6-24h)
Answer: We cultured for 96 hours. This was the optimal time-point to get the largest Th1 or Th2 response to antigen stimulation.

Results A again please consider through editing (English writing). Please delete redundant description of methodology or rationale. The results section should present just results only.
Answer: Yes. We agree. We have revised these parts.

Page 13
The results of DBPC oral challenge test
EFSS attenuated allergic reactions to food allergens in oral challenge test?
Some sentences are redundant and should be deleted A please just describe results only.
Answer: We have revised these sentences. The attenuation of allergy reactions to food allergens after the FESS could not be explained by the primary treatment as we demonstrated in the text. The mechanism might be that removal of CRS-derived SEB modulated the aberrant immune responses in the intestine. As the results show, SEB is required to sustain the antigen specific Th2 cell function. Once pathogens removed, self adjustment or other mechanisms promote intestinal immune homeostasis: less Th2 cytokine production, less IgE antibody generation and less mast cell sensitization.

Page 14
Serum IFN-I^3 levels: please check the scale of Fig. 1-C. Normal control healthy individuals seldom reveal IFN-I^3 levels higher than 50 pg/m; usually 1-20 pg/ml in most of the papers published.
Answer: That is the results. We would like to demonstrate it as is.

Page 15
Antigen specific T cell responses: More precise, accurate description of results is recommended such as written below:
When cultured with food allergens or SEB, PBMCs from CRS-FA patients produced higher levels of IL-4 (Fig. 3-A) than cells cultured without stimuli. PBMCs with stimulated with food allergen plus SEB revealed the highest IL-4 production. PBMCs from FA patients also revealed increase in IL-4 production with food allergen. However, FA PBMCs were less responsive to SEB than CRS-FA cells and concurrent cultures of SEB+food allergen did not further augment IL-4 production in FA patients.
Answer: Yes. We have revised this paragraph.

Intracellular cytokine staining: Intracellular cytokine staining does not necessarily directly reveal T cell proliferative responses. It is more accurate to say that these assays reflect frequency of IL-4 or IFN-I^3 expressing cells.
Answer: Yes. We agree. We have revised this part.

The results seem to indicate that CRS-FA PBMCs reveal significant increase in IL-4+ cells (if double stained with CD4, it can be described as IL-4+ CD4+ cells) with SBE+food allergen than SBE or food allergen alone. There might be a typo-error in % of IL-4+ cells in FA PBMCs following SBE stimuli; it looks like that SBE substantially decreased frequency of IL-4+ cells.
Answer: We have revised this paragraph. When PBMCs cultured in antigen or SEB alone, the IL-4 production increased first, then attenuated, could not sustain in contrast to the PBMCs cultured in the presence of both SEB and antigens.

Page 16
The first few sentences should be stated in the method section or footnote of Table 6. I assume that Table 6 present the data obtained from CFS-FA patients but it is unclear how reduction of symptoms scores is calculated. Please clarify.
The symptom scores were sum of individual symptom score. We use the differences of symptom scores after the sinus surgery.

Discussion aE"Again please consider further editing in English writing. Multiple grammatical errors are noticed and several sentences need to be rewritten for better clarification.
Answer: Yes. We have done.

Page 17:
1st paragraph; Simply summarize the conclusion of results. Description of mechanisms of action of SBE is redundant in the introduction. Please simplify. It is better to concentrate on describing how SBE contained in sinus secretion can potentially affect gut mucosal immune responses.
Answer: We have simplified the SEB portion in the introduction. The second question, "how SEB contained in sinus secretion....." has been described in the discussion.

Page 18
1st paragraph: Weal formation is generally regarded to reflect rapid increase in vascular permeability triggered by histamine. It is not clear how wheal formation quantitatively correlates with IgE levels bound to mast cells. Recommend simplifying the sentences just describing that FESS appeared to have significantly attenuated prick skin test reactivity to food allergens. It is ideal to test if there is any change of reactivity to other aeroallergens, since SBE in the upper airway can also potentially aggravate prick skin test reactivity to common aeroallergens such as dust mite.
Answer: Yes. We have revised this part.

2nd paragraph:
Partial relieve of the clinical symptoms A you mean partial relief?
This paragraph is not well structured. Revising the sentences will be helpful just concentrating on the results supporting the effects of FESS for attenuation of FA.
Answer: We have revised this part.

Page 19
1st paragraph. All the data indicates colonization of Staph. aureus. It is unclear if it can be said as infection.
Answer: Yes. We have added "infection" to where it should be.

2nd paragraph. It is better to describe that SBE can cause polyclonal T cell activation and depending on the pre-existing inflammatory condition, either Th1- or Th2- mediated inflammatory condition can be aggravated.
Answer: We have added this information into the text.

Last sentences: IL-5 does not facilitate IgE production.
Answer: Yes. We have deleted IL-5 from the text.

Page 20
1st paragraph. It is better to simply emphasize the results which indicate attenuation of Th2 responses to food allergens. Then should point out the possibility that this improvement can be associated with reduction of Staph. colonization after FESS.
Answer: We have revised this part.

2nd paragraph and continued discussion with Treg cells on Page 21. Discussion is unclear. Lack of the effects of SBE on PBMCs from FA patients does not support their speculation. These CRS+FA patients may have genetic predisposition to respond rigorously to SBE and by bystander effect, their Th2 responses to food allergens may be further augmented as one possible explanation. There may be other possibilities as well. This portion of discussion is hard to follow.
Answer: We have revised this part.

Page 21
2nd paragraph A it should be placed in the first part of discussion or in the introduction.
Answer: We have moved this part to the first part of discussion.