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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Version: 2 Date: 26 May 2006

Author's response to reviews:

Dear Editors:
The questions about my paper are answered below each question in capital letters
Thanks a lot.
Pingchang Yang

Reviewer's report

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

Reviewer: Harumi Jyonouchi

Reviewer's report:

General
This paper addresses important clinical questions prospectively whether sinus inflammation in association with Staphylococcal colonization aggravated 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.

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. WE HAVE ADDED THE INFORMATION TO THE FOURTH PARAGRAPH.

* 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.)
A TABLE (TABLE 1) HAS BEEN ADDED WITH THE DEMOGRAPHIC INFORMATION OF THE SUBJECTS IN THIS PAPER (THE REST TABLES' NUMBERS HAVE BEEN CHANGED). IN THE ORIGINAL STUDY DESIGNE, WE EXCLUDED THOSE WITH ALLERGIC HISTORY, THUS, NO ALLERGIC SUBJECTS WERE RECUITED IN THE GROUPS OF HEALTHY AND CRS.

* 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.

1, YES. WE AGREE, IT IS POSSIBLE THAT THERE ARE DIFFERENT T CELL CLONES EXIST IN THE BODY OF THESE SUBJECTS: (I) SEB SPECIFIC T CELL CLONE; (II) FOOD ANTIGEN SPECIFIC T CELL CLONE. THESE CLONES HAVE BEEN WELL DESCRIBED IN TEXT BOOK. OUR OBSERVATION CONFIRMED THE PHENOMENON AND FURTHER MORE. WE ALSO OBSERVED SOME T CELL CLONES ARE OVER RESPONDED WHEN EXPOSED TO BOTH SEB AND FOOD ANTIGENS. WE ACTUALLY HAVE NOT UNDERSTOOD THE NATURE OF THESE T CELL CLONES, BUT IT IS WORTH TO FURTHER EXPLORE THEIR IMMUNO-PATHOLOGICAL SIGNIFICANCE IN THE FUTURE.

2, ACCORDING TO LITERATURE, THE VALUE OF IFN-GAMMA ARE IS QUITE VARIABLE AMONG DIFFERENT PAPERS. DIFFERENT SOURCES OF SAMPLES, SUBJECTS AND REAGENTS MAY CONTRIBUTE TO IT.

Page 16, Fig 4. Likewise, Response to SBE may be just reflecting stimulation of SBE specific Th2 cells and resultant release of IL-4.

WE HAVE SOME MORE DATA FOR DEMONSTRATING THE EFFECT OF SEB PROMOTING TH2 RESPONSE IN THESE PATIENTS. HERE, WE ADDED THE NEW DATA REPLACING THE PREVIOUS FIG 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.

I WORKED IN CLINIC FROM 1986 TO 1996 AT ENT DEPARTMENT OF SHANXI MEDICAL UNIVERSITY OF CHINA. WE HAD AN ALLERGY CLINIC THAT WAS VISITED BY PATIENTS WITH ALLERGIC DISEASES. I NOTICED THAT MANY PATIENTS CLEARLY DESCRIBED THAT THEIR ALLERGY STARTED FROM AN INFECTION. I REALIZED THERE MIGHT BE A CONNECTION BETWEEN INFECTION AND ALLERGY. IN MY PHD THESIS, I DEVELOPED ANIMAL MODELS OF ALLERGIC RHINITIS AND ASTHMA. MICROBIAL PRODUCTS (I USED PERTUSSIS TOXINS) WERE REQUIRED TO BE ADJUVANTS IN ESTABLISHING ALLERGIC ANIMAL MODELS THAT IS ALSO WRITTEN IN TEXT BOOKS. RECENTLY, I OBTAINED SOME INTERESTING DATA. WHEN BONE MARROW GENERATED DENDRITIC CELLS ARE EXPOSED TO EITHER SEB OR OVA, THEN COCULTURE WITH NAIVE CD4+ T CELLS, SIGNIFICANT TH1 AND TH2 RESPONSES ARE GENERATED, BUT ONLY REMAIN HIGH LEVELS ABOUT 3 DAYS AND DECLINE QUICKLY AFTERWARDS. HOWEVER, WHEN DENDRITIC CELLS EXPOSE TO BOTH SEB AND OVA CONCURRENTLY, COCULTURE WITH NAIVE CD4+ T CELLS, IT EVOKES POLARIZED TH2 RESPONSE THAT LAST FOR MORE THAN 2 WEEKS. THESE IMMUNE RESPONSES CAN BE BLOCKED BY PRETREATMENT WITH TIM1 OR TIM4 shRNA. WE HAVE OBSERVED THAT DENDRITIC CELLS CAN CAPTURE BOTH SEB AND OVA INTO THE CYTOPLASM RESULTING IN INHIBITION OF IL-12 GENE EXPRESSION.

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
- 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.'
- THE FIRST TWO SENTENCES HAVE BEEN DELETED. THE THIRD SENTENCE HAS BEEN CHANGED
To "SEB is a potent immunomodulator and implicated with pathogenesis of inflammatory diseases mediated by Th1 or Th2 dominant immune responses"

**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.

**Results**
Not clearly described. I am not certain if I can completely agree with their interpretations (See Major Compulsory Revision section)

**Conclusion**
Better to rewrite to reflect the results a little more accurately.

* Introduction - better to rewrite in general.

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.

"life threaten" HAS BEEN REVISED TO "life-threatening".

WE HAVE REPLACED THE SECOND SENTENCE WITH "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."

THE FOURTH SENTENCE IN PAGE 3 WAS ABOUT MEMORY T CELLS THAT HAS BEEN DELETED.

WE REVISED HERE AS "some polyposis...".

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.

WE HAVE REVISED THESE TWO PARAGRAPHS.

**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.

WE HAVE REVISED THE LAST SENTENCE HAS BEEN RE-WRITTEN.

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.

YES. WE CHOSE ANTIGENS TO DO ORAL CHALLENGE BASED THE POSITIVE PRICK SKIN TEST THAT WAS DEMONSTRATED IN THE TEXT.

Page 9, SBE measurement paragraph: this part can be shortened substantially.

**Conclusion**
YES. WE AGREE. IF CD4+ T CELLS WERE ISOLATED AND THEN TO DO THE PROLIFERATION ANALYSES, RESULTS MAY BE MORE PRECISE. IN OUR OBSERVATION, WE USED PBMC TO OBSERVE TH2 RESPONSE TO SPECIFIC ANTIGEN STIMULATION THAT ALSO HAS ADVANTAGE, SUCH AS ANTIGEN PRESENTING CELLS NATURALLY MIX WITH CD4+ T CELLS, MORE CLOSE TO NATURAL ENVIRONMENT. TH2 CELLS ARE THE MAJOR IL-4 PRODUCERS IN PBMC. THUS, THE
RESULTS PRECISELY REFLEX TH2 RESPONSE IN RESPONSE TO SPECIFIC ANTIGEN STIMULATION.

* Results; Each paragraph tends to contain sentences associated with methods and such redundancy should be eliminated. THESE SENTENCES HAVE BEEN DELETED.

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. YES. WE AGREE. THIS PARAGRAPH HAS BEEN INCORPORATED INTO THE METHOD SECTION.

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. WE DID NOT REALIZE THIS POINT BEFORE. BUT LET IT AS IS.

Page 15, Staphylococcus aureus cultures; it is necessary to state how many CRS patients without FA revealed positive Staphylococcal cultures and degree of colonization. WE HAVE ADDED S. AUREUS DATA FROM PATIENTS WITH CRS ONLY TO FIG 2C.

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. WE DETECTED IL-4 TO PRESENT TH2 RESPONSE AND IFN- TO PRESENT TH1 RESPONSE THAT IS LISTED IN TABLE 5. WE HAVE ADDED THIS EXPLANATION TO THE NOTE OF TABLE 5.

Reviewer's report
Rhinosinusitis derived Staphylococcal enterotoxin B plays a possible role in the pathogenesis of food allergy
1 17 May 2006 Version: Date:
Francesca Levi-Schaffer Reviewer:
Reviewer's report:
General
The methodology is at the standards of the state of the art. The methodology description is also clear and well-written. In conclusion, the methodology is fine.

WE AGREE. THANKS.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
WE HAVE DONE SOME CORRECTIONS.

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

Discretionary Revisions (which the author can choose to ignore)
Declaration of competing interests:
I declare that I have no competing interests