Author's response to reviews

Title: Significant cross reactive antibodies to influenza virus in adults and children during a period of marked antigenic drift

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Version: 3 Date: 1 May 2014

Author's response to reviews:

1.5.2014

Dear Editor,

We were very happy to read that you are willing to see a revised manuscript. I am happy to tell you that we have revised our manuscript in complete accordance with the reviewers and the editors comments (changes are marked in red). Below please find our point by point response to the editors and the reviewers. I hope that you will now accept this manuscript for publication.

Best wishes,

Michal

Point by point response

Editor's Comment:

I would highly advise the authors to majorly address the two comments below brought up by the reviewers:

Comment: 1. The authors need to clarify the vaccination history of the subjects recruited in the study, specifically for the children. This will directly impact their conclusion as the authors is trying to correlate the cross-reactivity with natural exposure which means natural infections in other words. However, the annual flu vaccination could definitely play a role in producing cross-reactivity. The authors may need to rethink of or reword the title of the manuscript without the
appropriate information of immunization record.

Response: The children were not vaccinated. We now mention this on pages 8 and 11. We also reword the title in accordance with the suggestion made by the editors and reviewer 2 (Dr. Feng Liu).

Comment: 2. The authors need to be cautious and refer to some literatures when describing the antigenic similarity of circulating H3N2 viruses between 2003 and 2007. I would suggest the authors to read a few more related publications and do a HA sequence comparison for these H3N2 strains, especially for those antigenic sites on the head domain of HA. By this way, the authors could have a better understanding of the antigenic drift. It could also help the authors to discuss the potential mechanism of cross-reactivity observed in the sera of children.

Response: As requested we compare the sequences between the various viruses by using a phylogenetic tree to demonstrate the similarity and the differences between the viruses studied here. The phylogenetic tree which clearly demonstrate the differences between A/Panama and A/Wisconsin is now presented in new figure 1. We also mention the phylogenetic tree in the results and in the discussion sections (highlighted in red). In addition we have added several references indicating that the two major viruses analyzed here are indeed antigenically different.

Reviewer 1
Reviewer's report
Title: Significant cross reactivity to influenza virus in adults and children following natural exposure during a period of marked antigenic drift
Version:2Date:14 March 2014
Reviewer:Angel Porgador
Reviewer's report:
Comment: In this manuscript the authors investigated the antibody cross reactivity against influenza viruses (H3N2). The investigation was performed over a period of 5 years because during these years a major change in the type of influenza viruses circulating in Israel was detected. The authors demonstrated that they could detect antibodies that cross-react against two different strains of influenza both in children and in adults. While the cross reactivity that observed in adults is not surprising as they might have been exposed to other influenza virus strains during their life, the cross reactivity observed in children is very surprising as they were never exposed to the virus. It is an important investigation that demonstrates for the first time the existence of naturally occurring cross-reactive antibodies. I have only minor comments:

Response: We thank the reviewer for the constructive criticisms. We have modified the manuscript in complete accordance with the reviewer criticisms (changes are marked in red).

Comment: 1) The manuscript should be edited by a native English speaker
Response: The manuscript was edited by a native English speaker as requested.

Comment: 2) The discussion should be more focused
Response: We have modified the discussion as requested

Comment: 3) More details should be given in the methods section
Response: We now describe the methods in more details.

Comment: 4) Can the authors give details about the general immunization regime in the Israeli population
Response: We provide the requested details. Please see pages 8 and 11

Comment: Level of interest: An article of outstanding merit and interest in its field
Response: No response is needed here

Comment: Quality of written English: Needs some language corrections before being Published.
Response: The manuscript was edited by a native English speaker

Comment: Statistical review: No, the manuscript does not need to be seen by a statistician.
Response: No response is needed here

Comment: Declaration of competing interests:
'I declare that I have no competing interests'
Response: No response is needed here

Reviewer's report

Title: Significant cross reactivity to influenza virus in adults and children following natural exposure during a period of marked antigenic drift

Version: 2 Date: 14 April 2014

Reviewer: Feng Liu

Reviewer's report:

Comment: Mandelboim et al. reported their findings to demonstrate significant cross reactive (CR) antibodies to influenza virus in adults and children following natural exposure during a period of marked antigenic drift. The findings bear good scientific merit and will contribute to better understand the mechanism of naturally occurred cross reactive antibody in human population following infection of flu virus. A few points need to be addressed and some revisions/rewarding are necessary before further consideration of publishing.
Response: We thank the reviewer for the constructive criticisms. We have modified the manuscript in complete accordance with the reviewer criticisms (changes are marked in red).

Major Compulsory Revisions

Comment: 1. What’s the flu vaccination history of these subjects recruited in this study, especially for those children? The authors emphasized natural flu infection, but they didn’t provide any evidence or statement that those children were not vaccinated yet when they were taken blood draw. This is an important part of experiment design, otherwise the authors cannot say the CR antibodies detected were purely induced by natural flu infection.

Response: This is an important comment and we apologize for not mention it before. The children studied here were not vaccinated. We now mention this on pages 8 and 11.

Comment: 2. Results, the second paragraph. The authors can only say “the A/Wyoming virus are antigenically similar to A/Fujian” but NOT the A/California virus. (No. 8, 2005, 80, 65–76 http://www.who.int/wer). In addition, A/Wisconsin/67/05 strain is also NOT antigenically similar to A/Fujian/411/02.

Response: We now provide phylogenetic tree to demonstrate the resemblance and the difference between the various viruses studied here. Please see new figure 1 and text.

Comment: 3. Discussion. The fifth paragraph, the authors cannot say “…thus these CR antibodies appear to be protective” because we can only refer to the threshold of HI titer of #40 as an indication of reduction of 50% risk from flu infection. To be cautious, I would suggest to reword the sentence as “…might provide protective function.”

Response: We modified this sentence as suggested.

Comment: 4. Discussion. The first half of sixth paragraph is quite confusing and not clearly delivering the authors’ point to me. Suggest to reword it. In addition, the same as comment 11 above, the authors cannot say “…as our detection assays requires that the generated antibodies will be protective.” Again, suggest to change it as “we use the threshold of HI titer of #40 as a positivity.” Again, the authors need to revise this part.

Response: We modified this sentence as requested.

Minor Essential Revisions

Comment: 1. The authors should add line numbers cross the manuscript to make tracking easier.

Response: We have added the line numbering as requested.
Response: We modified the title as suggested by the reviewer and the editors.

Comment:3. In Abstract, Results part, the authors need to clearly state that 13% in 2002 and 29% in 2007 of sera from children shows HI antibodies titers of # 40 against both Panama/1999 and Wisconsin/2005. The current description is confusing.
Response: We modified the sentence as suggested.

Comment:4. Methods, Sample collection. It’s indicated that samples were collected from children aged 1-17 yr here, but only those from aged 1-3 yr were used in the study, right? Change “age 1-17” into “age 1-17 years”, and the same for age 1-3.
Response: We modified the sentence as suggested.

Comment:5. Methods, HI assay part. The last sentence, change “…active since 2006.” into “active in 2006-2007 season.”
Response: We modified the sentence as suggested.

Comment:6. Discussion. The first paragraph, suggest to move “following 4 years of infections” after “…strain A/Panama/2007/99”.
Response: We modified the sentence as suggested.

Comment:7. Discussion. The third paragraph, change “…2009 swine influenza…” into “…2009 swine origin influenza…”.
Response: We modified the sentence as suggested.

Comment:8. Discussion. The fourth paragraph, the mortality rate of H5N1 human infection is about 60%, but not 50% (see WHO webpage).
Response: We modified the sentence as suggested.

Comment:Level of interest: An article whose findings are important to those with closely related research interests
Response: No response is needed here.

Comment:Quality of written English: Needs some language corrections before being published
Response: The manuscript was edited by a native English speaker.

Comment:Statistical review: Yes, and I have assessed the statistics in my report.
Response: We refer to these above.

Comment: Declaration of competing interests: I declare that I have no competing interests.
Response: No response in needed here.