Author's response to reviews

Title: Does Glycosylation as a modifier of Original Antigenic Sin explain the case age distribution and unusual toxicity in pandemic novel H1N1 influenza?

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Author's response to reviews: see over
Dear Editor,

I am pleased to enclose our revised contribution: “Does Glycosylation as a modifier of Original Antigenic Sin explain the case age distribution and unusual toxicity in pandemic novel H1N1?” (MS: 1690646193303755) as a Debate article for consideration for publication in BMC Infectious Diseases. We have submitted the earlier version to BMC Medicine, and are now submitting the revised version to BMC Infectious Diseases following the interim decision.

Revisions were made following the reviewers’ suggestions; point-by-point responses are provided in this enclosure.

We hereby approve of the revised manuscript and declare that the manuscript has not been considered for publication elsewhere. We also declare that we have no conflicts of interest. We hope the revised manuscript is now suitable for publication.

Yours sincerely,

Tom Reichert
[--------Response to the Reviewer 1--------]
The authors of the submitted manuscript created a composite age distribution of confirmed cases of novel H1N1 through mid- to late-July for 10 countries on five continents (Shown in Figure 1). Based on the composite, Reichert et al. indicated that over 75% of confirmed cases of novel H1N1 occurred in persons < 30 years old, and less than 3% of cases occurred in persons over 65, with a gradation in incidence between ages 20 and 60 years. The authors discuss that the explanation for this case age distribution is due to the doctrine of Original Antigenic Sin. The authors also noted that the novel H1N1 virus lacks glycosylation sites on the globular head of hemagglutinin (HA1) near antigenic regions, a pattern shared with the 1918 pandemic strain and H1N1 viruses that circulated until the early 1940s. Later H1N1 viruses progressively added new HA1 glycosylation sites likely to shield antigenic epitopes. The authors explain that this evolutionary context, Original Antigenic Sin exposure should produce an immune response increasingly mismatched to novel H1N1 in progressively younger persons. Thus, it is this mismatch that produces both the gradation in susceptibility to novel H1N1 and the unusual toxicity.

The point made in the submitted manuscript is the principle of Original Antigenic Sin, modified by glycosylation appears to explain both the case age distribution and the unusual toxicity pattern of the novel H1N1 virus. A conclusion made is that the ongoing pandemic is an extraordinary opportunity to validate, and possibly extend the doctrine of Original Antigenic Sin and that these studies also point to widely available pharmaceutical agents as plausible candidates for mitigating the toxic effects.

The data put forth on the composite age distribution (Figure 1) and the glycosylation of H1N1 viruses (Figure 2) are well performed and advance the knowledge in the field. However, the theological concept that Original Antigenic Sin explains the case age distribution is conjecture. Although the manuscript is grammatically well-written, the discussion on the original antigenic sin is not convincing. The authors failed to mention that the Sa and Sb antigenic sites, located near the top of the globular head of H1 HA of 2009 viruses show strong conservation of the 1918 sequence. In contrast, the 2009 seasonal H1 HA show little antigenic identity to the 2009 H1N1 viruses. Thus, older individuals that generated an antibody response to the Sa and Sb
antigenic sites would have neutralizing antibodies to the 2009 virus. Such a cross-reactive antibody response supports epidemiological findings in the 2009 pandemic that people over about age 60 may have a degree of immunologic protection independent to the phenomenon of 'original antigenic sin'.

Our response: We agree with the reviewer that immunity to similar viruses encountered in early childhood is responsible for the protection of older individuals from this virus – indeed, we believe this is the very definition of OAS – the immune responses to their original antigenic encounter now provide these persons cross-protective immunity to those and similar viruses (but not to viruses which are not closely related). We have attempted to build the case through phylogenetic analysis that the H1N1 S-OIV is indeed closely related to those viruses which circulated in the 1930s and 1940s, when older individuals would have had their “original” encounter. Since we obviously failed to make this point clear enough for the reviewer, we have modified this section to better explain our reasoning. We have also inserted additional lines of evidence from the epitope comparisons made in the Tumpey paper referred to in the review, from an older paper giving more detail on the components of antigenic regions on HA1, and from a recent analysis of B-cell and T-cell epitopes all of which support our contention that this virus will appear to the immune system to be more similar to older viruses than to recent seasonal strains. We intended no theological implications, plead congenital irreverence, and point out that reference to the colorful phrase, Original Antigenic Sin continues to be widespread (cf. reference #4 in Fisman DN, Savage R, Gummay J, etal. Older age and a reduced likelihood of 2009 H1N1 infection. N Engl J Med 12 November 2009;361(20):2000-1).

Specific comments:
1. Page 8, paragraph 1. I think the authors meant to insert reference #24 after the sentence, “As a counter-balance….lower respiratory tract”.

Our response: We have added this reference (now renumbered) in this location as requested.

2. Page 9, second paragraph, “response” is misspelled.

Our response: We have corrected the typo, “resonsse” in the third paragraph on p. 9

3. Page 12, first bullet, “throughout” is misspelled.
Our response: The neglected “t” has been restored.

[-------Response to the Reviewer 2-------]

In this manuscript, the authors speculate over the observed age pattern of H1N1 cases reported to national authorities in several countries. The interpretation is based on the elevated portion of cases among young adults; together with the changing glycosilation profile of H1N1 viruses through time and the original antigenic sin. As the authors acknowledge in the manuscript, a large part of the age distribution may be the result of either biased sampling (see for example the data for Japan where this is said in Nishiura's Eurosurveillance paper) or initial distribution of cases due to increased contact within the young age classes and school closure for the youngest. It seems to be common in pandemics (see flucurrents Shweta The Shifting Demographic Landscape of Influenza;http://knol.google.com/k/the-shifting-demographic-landscape-of-influenza). In 1957 the age patterns were similar (lower in the very young and the old, larger in the young adults, see for example Dunn Am J Hyg 1959). Therefore, one may question the assertion by the authors p 10 ("...we believe there may be additional immunological factors").

Our response: The reviewer is quite correct in noting that a shift in age-distribution to younger age with apparent “reductions” in mortality in the very young and very old is a common feature of influenza pandemics. Such observations have many layers, however. First of all, the age distributions of cases, hospitalizations and mortality all differ from each other in detail both within pandemics and across the four pandemics that have occurred in the last century. The Shweta knol referenced above, for example, found the 1968 pandemic to be different from the other three pandemics at the case level. Beyond this it is important to note that their work does not dissect the pediatric age distribution beyond a focus on “school-age” children. Other and earlier work suggests that influenza epidemics and pandemics should be led, in time and age-specific case rates, by the pre-school subpopulation (ages 3-4) (c.f. Brownstein JS, Klienman KP and Mandl KD, Identifying pediatric age groups for influenza vaccination using a real-time regional surveillance system. Am J Epidemiol 2005;162:686-93.) We discuss in this paper only the case age distribution of the novel H1N1 and find a distinct excess in that distribution in the 10-19 age group. Therefore, while it may be that an unusual transmission characteristic of novel H1N1 has operated to favor the social contact structure of 10-19 year old children over
pre-school children, it does not seem unreasonable to seek other effects in immunological factors as well.

We thank the reviewer for the suggestion and have altered our text accordingly (Page 10 Line 1).

If the attack rate is indeed larger in the young, there is uncertainty regarding the actual severity: large increase in severe pneumonia in young people –Chowell NEJM 2009, but larger mortality in those aged over 50 years after hospitalization - Louie JAMA 2009.

Our response: Evidence in Mexico (Echevarria-Zuno et al. Lancet 2009; in press) suggests that the case fatality ratio (conditioned on confirmed cases) is seemingly regulated by presence or absence of underlying disease (and thus, the age-specific case fatality ratio increases as a function of age). Thus, the increase in severe pneumonia in young is likely to be proportional to the increase in cases, while at the same time the large mortality among those over 50 years derives from the increased proportion with underlying medical conditions.

The proposed explanation is pleasant, involving evolutionary arguments regarding the increasing level of glycosylation of the flu virus. It is not clear if/how this could be validated. Part of the supporting evidence is still unpublished (sequential infection in mice).

Our response: We added brief suggestions of possible mechanisms to validate our extensions of OAS (P12)

We greatly appreciate the above comments of the reviewers and are certain that this response has helped us improve this manuscript. We hope the critique has been appropriately addressed and that the manuscript is now suitable for publication.