Adaptation of avian influenza A (H6N1) virus from avian to human receptor binding preference

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Review timeline:

Submission date: 06 January 2015
Editorial Decision: 04 February 2015
Revision received: 23 March 2015
Editorial Decision: 08 April 2015
Revision received: 10 April 2015
Accepted: 16 April 2015

Editor: David del Alamo

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 04 February 2015

Thank you for the submission of your manuscript entitled "Adaptation of avian influenza A (H6N1) virus from avian to human receptor binding preference". We have now received the reports from the referees, which I copy below.

As you can see from their comments, both referees support the publication of your study in The EMBO Journal, but point out to a number of concerns, in particular referee #2, that will require your attention before we can accept your manuscript. I think these issues, mostly involving further discussion and clarifications in the case of referee #1, and involving further experimental evidence in the case of referee #2, are fairly clear and reasonable but please do not hesitate to contact me if you have any questions, need further input on the referee comments or if you anticipate any problems.

As a matter of clarity, I would like to explicitly point out to a concern raised by both referees with regards to references. Please remember that references in all EMBO publications are unlimited in number and FREE OF CHARGE for the author, in order to favor primary literature citation.

In light of this assessment, I would like to invite you to submit a revised version of you work. We normally allow a single round of major revision only, which should be submitted within the next three months. Should you foresee a problem in meeting the three-month deadline, please let us know in advance and we may be able to grant an extension.

I would also like to point out that as a matter of policy, competing manuscripts published during this
period will not be taken into consideration in our assessment of the novelty presented by your study ("scooping" protection). However, we would appreciate if you contact me as soon as possible upon publication of any related work in order to discuss how to proceed.

When preparing your letter of response to the referees' comments, bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process initiative, please visit our website: http://emboj.msubmit.net/html/emboj_author_instructions.html - a2.12

As you have probably seen already, every paper now includes a 'Synopsis', displayed on the html and freely accessible to all readers. The synopsis includes a 'model' figure as well as 2-5 one-short-sentence bullet points that summarize the article. I would appreciate if you could provide this figure and the bullet points.

Finally, in order to ensure good reporting standards and to improve the reproducibility of published results, our guidelines to authors are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Accordingly, we now require the submission of a completed author checklist, which covers in a systematic manner your practices regarding animal welfare, human subjects, data deposition and research ethics. It needs to be filled (not all fields may apply to your study in particular) and returned to the editorial office at revision, either via the online submission system as a supplementary file or by email (contact@embojournal.org). Please, click on the link below and follow the instructions to download the checklist file:

http://emboj.embopress.org/authorguide

Thank you very much again for the opportunity to consider your work for publication. I look forward to your revision.

REFEREE REPORTS

Referee #1:

The paper by Wang et al. addresses important gaps in our knowledge about the evolution of receptor binding preference in influenza virus. Receptor binding contributes to host tropism and potentially to the emergence of new strains in humans. The results presented in the paper are novel and scientifically sound. The authors identify residue alterations in the HA of H6N1 influenza that alter HA's receptor specificity in a way that favors human vs. avian host tropism, and provide structural explanations for the altered receptor binding. The binding studies, and the structural studies, including resolution of HA free and bound to the receptor analogs, are terrific and will be of interest to virologists who study virus-host interaction and/or viral entry.

The paper has flaws that make it difficult to appreciate its content.
1. The paper focuses on the avian H6N1 and its potential for adaptation to humans. The authors overstate the medical importance of H6N1 given the absence of human infection (1 case) and lack of human-to-human transmission. These statements in the introduction and discussion should be extensively tempered. It should be made clear that the focus is on understanding a contribution to the mechanism of host switching, focusing on the role of HA in that process, with the example of H6N1. The likelihood of H6N1 becoming a significant human pathogen is overstated and it is important to temper this appropriately. (Note, the significance of the work lies in its mechanistic clues and its scientific value, and does not rest on the medical importance of the specific virus studied.)

2. While mention is made in the introduction of the fact that the influenza viral replication cycle is a result of a balance between the two glycoproteins- HA and NA- of the virus, this aspect of the evolution of receptor specificity / host tropism is then inadequately addressed. The studies adopt an exclusive focus on HA and mutations in HA in three strains of H6N1 (avian and "human"). This approach provides useful data however there needs to be serious attention to the fact that in the host tissue the receptor binding HA is not acting in isolation but in a direct balance with the receptor-cleaving NA. It is really hard to draw conclusions based on changes in HA without looking at
whether there are corresponding, or cooperating, or compensatory, changes in NA. In this light, the authors need to be more careful about drawing conclusions about what may happen in vivo. This comment does not negate the value and rigor of the HA-focused studies, however the context must be made clear.

3. In general, the results need to be better placed in the context of the existing literature on host tropism and the HA/NA balance.

4. The way the results section is written makes it difficult to appreciate the work that has been done. The results paragraphs would benefit from restructuring so that the reader can more readily grasp the relevant conclusions in each section (delineate the specific question, the experiment, and the conclusion). There is a mixture of results, background, discussion, and speculation in each part of the results that make it harder to appreciate the actual results.

5. The other element that detracts from appreciation of the data is the presentation of figures 1 and 2. The figures themselves should be completely redone - the lettering is too small to read and it would be easy to remake these so that they are legible. More importantly the figures need to be described clearly in the text (see issue with results section above). The structural figures are a pleasure to study and the tables are acceptable.

Minor points.

1. Mention is made in the first paragraph of the introduction of the fact that the influenza viral replication cycle is a result of a balance between the two glycoproteins - HA and NA - of the virus. The balance between HA and NA has been discussed extensively in the literature and the authors of this manuscript need to reference this statement (for example, Klenk group papers - Ohuchi, Wagner etc).

2. The paper requires some editing for grammar and usage throughout.

3. The paper needs better referencing overall - the authors have used a somewhat selective set of references and need to be significantly more cognizant of the field.

Referee #2:

For the authors:

In this manuscript, Fei Wang and colleagues analysed the binding preference and structure of HA of human and avian H6N1 influenza viruses. The authors conclude that evolution of H6N1 viruses in Taiwan went through three main stages, from avian receptor binding preference, to dual receptor specificity, to a preference for human receptors. Structural analyses suggest that a single amino acid substitution, P186L, was responsible for the switch from the second to the third stage.

Although the paper is of interest, a major shortcoming is the incompleteness of the analyses. First, the molecular and structural determinants of the switch from avian receptor specificity to dual receptor specificity was not investigated. Given that the majority of strains that are currently circulating lack the P186L substitution, this is a major omission. In my opinion - and in agreement with the overall structure of the manuscript - it would be appropriate to investigate the effect of mutations at positions 186-190-226-228, going from P-E-Q-G to P-V-Q-S to L-V-Q-S in the same HA background. On the latter point, it is important to note that - because there are more amino acid substitutions between cH6-HA and hH6-HA than P186L alone - , we can never be sure that the structural changes that were observed were indeed due to P186L (in particular given the fact that P186L did not interact with the receptor directly). Therefore, I am of the opinion that further experiments are required to reach solid conclusions.

Additional comments:

The reference list needs some attention. Perhaps there is an alternative for the 1978 Russian H1N1 reference? Also, authors should balance the reference list better, including more references to original works, and less overlapping references to own work. A good example is the 7 references to bat HA/NA, and 0 references for the subsequent sentence on HA/NA balance. This also applies to other parts of the manuscript.
Is it inappropriate to speak of a fourth emerging stage with n=1 case (page 9).

The paragraphs at the bottom of page 10 and top of page 11 contain no data; this is only citation of the literature and should be removed. Rather, it would be appropriate to show and describe the HA structures without receptor.

What are systematic diseases? (page 4). The entire paper could be improved by having it read by a native English speaker.

Table 1 can hardly be described as an "evolutionary analysis". Rate (footnote) is not an appropriate term here.

The legends to figures could be improved by avoiding duplications (e.g. Fig. 1), and by explaining everything that is shown (Fig. 1, e.g. colours). I think that titles in figures are not needed, if the right legends are provided. Figures 3 and 4 are essentially the same, just slightly rotated and other details highlighted? This could easily be shown in 1 figure.

1st Revision - authors' response 23 March 2015

Referee #1:

The paper by Wang et al. addresses important gaps in our knowledge about the evolution of receptor binding preference in influenza virus. Receptor binding contributes to host tropism and potentially to the emergence of new strains in humans. The results presented in the paper are novel and scientifically sound. The authors identify residue alterations in the HA of H6N1 influenza that alter HA's receptor specificity in a way that favors human vs. avian host tropism, and provide structural explanations for the altered receptor binding. The binding studies, and the structural studies, including resolution of HA free and bound to the receptor analogs, are terrific and will be of interest to virologists who study virus-host interaction and/or viral entry.

The paper has flaws that make it difficult to appreciate its content.

1. The paper focuses on the avian H6N1 and its potential for adaptation to humans. The authors overstate the medical importance of H6N1 given the absence of human infection (1 case) and lack of human-to-human transmission. These statements in the introduction and discussion should be extensively tempered. It should be made clear that the focus is on understanding a contribution to the mechanism of host switching, focusing on the role of HA in that process, with the example of H6N1. The likelihood of H6N1 becoming a significant human pathogen is overstated and it is important to temper this appropriately. (Note, the significance of the work lies in its mechanistic clues and its scientific value, and does not rest on the medical importance of the specific virus studied.)

Reply: We have revised the introduction and discussion by toning down as a human pathogen, and deleted the statements about the medical importance of H6N1. As the referee suggested, we now focus on the mechanistic clues of the receptor binding determinant of H6 subtype virus.

2. While mention is made in the introduction of the fact that the influenza viral replication cycle is a result of a balance between the two glycoproteins- HA and NA- of the virus, this aspect of the evolution of receptor specificity / host tropism is then inadequately addressed. The studies adopt an exclusive focus on HA and mutations in HA in three strains of H6N1 (avian and "human"). This approach provides useful data however there needs to be serious attention to the fact that in the host tissue the receptor binding HA is not acting in isolation but in a direct balance with the receptor-cleaving NA. It is really hard to draw conclusions based on changes in HA without looking at whether there are corresponding, or cooperating, or compensatory, changes in NA. In this light, the authors need to be more careful about drawing conclusions about what may happen in vivo. This comment does not negate the value and rigor of the HA-focused studies, however the context must be made clear.

Reply: Thanks for kind suggestion. We have added some discussion about the HA-NA balance related to host tropism. Please see in the discussion section “Moreover, it should be aware that, during the viral entry of the host tissue, the receptor binding HA is not acting in isolation but in a direct balance with the receptor-cleaving NA. Besides receptor binding of HA, HA-NA balance...
should also be taken into consideration when we evaluate the epidemic or pandemic potential of avian influenza viruses.”

3. In general, the results need to be better placed in the context of the existing literature on host tropism and the HA/NA balance.

Reply: Yes, we have restructured the result section and made it to read more smoothly. As discussed above, we also add comments about the HA-NA balance.

4. The way the results section is written makes it difficult to appreciate the work that has been done. The results paragraphs would benefit from restructuring so that the reader can more readily grasp the relevant conclusions in each section (delineate the specific question, the experiment, and the conclusion). There is a mixture of results, background, discussion, and speculation in each part of the results that make it harder to appreciate the actual results.

Reply: We have restructured the results section as the referee suggested and just described the results, removing all the related background, speculation and discussion.

5. The other element that detracts from appreciation of the data is the presentation of figures 1 and 2. The figures themselves should be completely redone- the lettering is too small to read and it would be easy to remake these so that they are legible. More importantly the figures need to be described clearly in the text (see issue with results section above). The structural figures are a pleasure to study and the tables are acceptable.

Reply: We have revised the figures 1 and 2, and remade the lettering so that they are legible. We also rewrite the results section and make sure that the description is clear.

Minor points.

1. Mention is made in the first paragraph of the introduction of the fact that the influenza viral replication cycle is a result of a balance between the two glycoproteins- HA and NA- of the virus. The balance between HA and NA has been discussed extensively in the literature and the authors of this manuscript need to reference this statement (for example, Klenk group papers -Ohuchi, Wagner etc).

Reply: As the referee suggested, we have referenced this statement in the first paragraph of the introduction.

2. The paper requires some editing for grammar and usage throughout.

Reply: We have asked a native speaker to help to edit the paper for grammar and usage throughout.

3. The paper needs better referencing overall - the authors have used a somewhat selective set of references and need to be significantly more cognizant of the field.

Reply: We have cited more references at the revised manuscript, avoiding to use a somewhat selective set of references.

Referee #2:

For the authors:

In this manuscript, Fei Wang and colleagues analysed the binding preference and structure of HA of human and avian H6N1 influenza viruses. The authors conclude that evolution of H6N1 viruses in Taiwan went through three main stages, from avian receptor binding preference, to dual receptor specificity, to a preference for human receptors. Structural analyses suggest that a single amino acid substitution, P186L, was responsible for the switch from the second to the third stage.

Although the paper is of interest, a major shortcoming is the incompleteness of the analyses. First, the molecular and structural determinants of the switch from avian receptor specificity to dual receptor specificity was not investigated. Given that the majority of strains that are currently circulating lack the P186L substitution, this is a major omission. In my opinion - and in agreement with the overall structure of the manuscript - it would be appropriate to investigate the effect of mutations at positions 186-190-226-228, going from P-E-Q-G to P-V-Q-S to L-V-Q-S in the same HA background. On the latter point, it is important to note that - because there are more amino acid substitutions between cH6-HA and hH6-HA than P186L alone - , we can never be sure that the
structural changes that were observed were indeed due to P186L (in particular given the fact that P186L did not interact with the receptor directly). Therefore, I am of the opinion that further experiments are required to reach solid conclusions.

Reply: Thanks for the referee’s good suggestion. We have performed mutagenesis experiments at positions 186-190-226-228 in the same background of chicken-H6N1 HA. As expected, we found that the E190V and G228S substitutions are determinants to confer the H6 HA with human receptor binding capacity, and the P186L substitution can dramatically reduce the binding to avian receptor. Please see the new section “Mutagenesis work on chicken-H6N1 HA” for more details.

Additional comments:
The reference list needs some attention. Perhaps there is an alternative for the 1978 Russian H1N1 reference? Also, authors should balance the reference list better, including more references to original works, and less overlapping references to own work. A good example is the 7 references to bat HA/NA, and 0 references for the subsequent sentence on HA/NA balance. This also applies to other parts of the manuscript.

Reply: Thanks for the referee’s suggestion. We have chosen an alternative for the 1977/78 Russian H1N1 reference, and referenced the sentence on HA/NA balance. Furthermore, we have adjusted the references in other parts of the manuscript.

Is it inappropriate to speak of a fourth emerging stage with n=1 case (page 9).

Reply: Considering referee 1’s comments, we toned down the expression of the potential public health significance. We didn’t use the phrase “a fourth emerging stage” any more, corrected as “a case of human infection”.

The paragraphs at the bottom of page 10 and top of page 11 contain no data; this is only citation of the literature and should be removed. Rather, it would be appropriate to show and describe the HA structures without receptor.

Reply: Yes, we have removed the paragraphs as the referee suggest and just described the overall HA structure.

What are systematic diseases? (page 4). The entire paper could be improved by having it read by a native English speaker.

Reply: Thanks for the referee’s suggestion. We have corrected the “systematic diseases” as “diseases”. And we also asked a native English speaker to help to improve the paper.

Table 1 can hardly be described as an "evolutionary analysis". Rate (footnote) is not an appropriate term here.

Reply: We have described the Table 1 as “Comprehensive analysis”, and corrected “rate” as “frequency”.

The legends to figures could be improved by avoiding duplications (e.g. Fig. 1), and by explaining everything that is shown (Fig. 1, e.g. colours). I think that titles in figures are not needed, if the right legends are provided. Figures 3 and 4 are essentially the same, just slightly rotated and other details highlighted? This could easily be shown in 1 figure.

Reply: We have improved the legend of figure 1, avoiding duplications. For titles in figures, we’d like to keep it to show the comparison more directly, when the readers view the figures at one sight. We have combined the figure 3 and 4 together in 1 figure, please see revised figure 4.

Thank you for the submission of your revised manuscript to The EMBO Journal and please accept my apologies for the delay in responding due to the recent holiday break. As you will see below, your article was sent to former referee #2, who now considers that you have properly dealt with the main concerns originally raised in the review process, and therefore I am writing with an 'accept in principle' decision, which means that I will be happy to formally accept your manuscript for publication once a few more minor issues have been addressed.

As I said, referee #2 now believes that all major concerns have been addressed and your manuscript is almost ready for publication (see below). Only one minor issue will still require your attention,
which refers to the citation and discussion of a recently published work. I would be grateful if you could address this small point.

Browsing through the manuscript myself I have also noticed a very small issue with data presentation: the statistical analysis of the results presented in figure 2 requires a more detailed description. As a guide, statistical analyses must include a definition of the error bars used and the number of independent experiments performed. In case a significance analysis tool is used, it should be specified as well.

If you have any questions or need any further input, please do not hesitate to contact me.

Thank you very much for your patience. I am looking forward to seeing the final version of your manuscript. Congratulations in advance for a successful publication.

REFEREE REPORTS

Referee #2:

The authors have addressed the issues that I raised in the previous round appropriately. Some grammar errors still exist, but these can presumably be handled by EMBO staff.

In the meantime, another manuscript described the structural analysis of the human H6 HA (Tzarum et al., Cell Host & Microbe 2015). Interestingly, the results of that study are contradictory to this one with respect to receptor preference. The present study describes numerous additional analyses that were not in the Tzarum paper, so the present paper is still a very valuable contribution to the field. Perhaps the authors would like to add a short paragraph (if EMBO J would allow), to address differences in the analyses of Tzarum et al. and Fei Wang et al., that could potentially explain the different outcomes of the two studies.