Reviewer's report

Title: Immune response to live-attenuated Japanese encephalitis vaccine (JE CV) neutralizes recent Japanese encephalitis virus isolates from South-East Asia and India

Version: 3  Date: 4 March 2014

Reviewer: Jun Luo

Reviewer's report:

In the present revision, the authors have revised some parts according to the reviewers’ comments. However, several important problems still exist in this MS, which may result in a suggestion of reject.

Major Compulsory Revisions

1) In Methods & Results, five different titer categories were created, including the increasing titers from low (titers 40-80, two pools) through medium (titers 160-320, two pools), high (titers 640-2560, two pools) and very high (titers >5120, one pool), I think at least three independent pools of each category should be included for evaluating their neutralization sensitivities to distinct JEV strains. The authors have stated that it is difficult to collect more clinical samples. However, it seems that some serious problems appeared in the subsequent RESULT.

2) Firstly, the authors selected individual serum samples for pooling was based on JEV neutralizing antibody titers determined in JE-CV PRNT50 assay previously as part of the study [22]. However, when they repeat this experiment again, the results of the serum PRNT50 against JE-CV is not accordant to their previous criteria for serum selection. For an example, as the authors shown in Table 2, PRNT50 of sample 7 (sera titer category of very high) against JE-CV is only 2865±? rather than >5120. Similar phenomenon exists in some of the other groups. Why?

3) Secondly, as shown in Table 3, the authors ranked the neutralization sensitivity of each sera pool to each JEV strain. However, it is strange that for any of the groups, low, medium and/or high of titers, none of a same or similar rank is observed in the two repeated pools of each group. Why? It is also strange that the neutralization sensitivities to JE-CV and SA14-14-2 is not the highest, but the serum are collected from JE-CV immunized children and the vaccine JE-CV is developed by replacing the pre-membrane and envelope coding sequences from the yellow fever vaccine virus (strain 17D) genome with the corresponding sequences from the SA14–14–2 virus strain [5, 6]. What is the reason and how to explain such a result?

4) For the problems mentioned above, the reviewer suggests that it may be
caused by the unsuitable designment of serum selection and grouping. The selection of serum and grouping performed by the author are used of mixed serum pools (with titers 1:40~1:80, 1:160~1:320, and so on) rather than the homogeneous serum samples (such as 1:40, 1:80, 1:160, 1:320, 1:640, and so on). May be this is more scientific for such a study.

5) For the Statistical methods, although the authors deleted the Table 4 and ranked the neutralization sensitivity for seven JEV strains within each sample pool using GMT in Table 3, an acceptable and scientific result has not been observed. With present data, it is no meaning to do such a comparison.

6) Finally for the results, figure 1 and table 2 are duplicated data, data in Table 3 is not solid and meaningful. Furthermore, previous studies have reported that the GIII JEV vaccines are protective for GI isolates. No enough novel finding is presented in the present revision. Thus, I still insist my opinion that a brief report or a short communication may be more suitable for publishing, accompany with a more scientific designment of experiments.

Minor Essential Revisions

1) Page 6, the first paragraph for introducing serum information is ambiguous and confusable for the readers, especially the “Each pool included 7–20 individual samples, all except two of which were from children who were serologically JE-naïve before vaccination (one subject in each of the low titer pools had a JE-CV PRNT50 titer of #10). All except 3 of the samples were also serologically dengue-naïve before vaccination (indicated by dengue PRNT titer #10 to at least one serotype; one subject in one low titer pool, one subject in one medium titer pool, and one subject in the very high pool”. Revision is needing.

2) Page 17, legends in figure 1, revise TVP-236 to TVP-8236?.

3) Page 17 & 19, figure 1 and table 2 is duplicated, and the data in Table 2 should be represent as mean ±SD or mean ±SE.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare no competing interests in relation to the paper. For the following questions, all of the answers are no and/or not.