Author's response to reviews

Title: A Human In Vitro Model System for Investigating Genome-Wide Host Responses to SARS Coronavirus Infection

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Version: 3 Date: 23 August 2004

Author's response to reviews:

23rd Aug 2004

Dear BioMed Central Editorial Team,

Thank you for your reply on the manuscript submitted. We noted the reviewers' helpful comments, and have addressed their comments below, revising the manuscript with improvements accordingly. The details of our response are given below:

MS: 8874017623968129 - A Human In Vitro Model System for Investigating Genome-Wide Host Responses to SARS Coronavirus Infection

Answers to Reviewers' comments

Reviewer 1:

Major Compulsory Revisions:

1. "It would be important to give information about the SARS-CoV strain used for infection and possibly compare it with other strains. In our experiments we were not able to obtain SARS-CoV replication in PBMCs, using the FFM1 and FFM2 strains."
   Ans: This has been addressed and the sentence "A seed stock of SARS-CoV (strain SIN 2774) passaged ir Vero E6 cells was used for infection" has been added on page 5, line 5. We believe that many experiments could be facilitated by the work presented here, including a large scale comparison of strains. We have also added another reference (Gillim-Ross et al., 2004) on page 8, line 11 indicating that another group was able to grow SARS-CoV in a range of cells.

2. "The effect of neutralizing antibodies from patient's serum has already been described. Therefore the SARS-CoV antibody binding experiment in this work gives no new information and could be omitted."
   Ans: Figure has been omitted and the text has been rewritten as "Antibody blocking experiments were also performed in which SARS-CoV was pre-incubated with convalescent patient sera for 30 minutes before introduction to the PBMCs and after a 4 day incubation period, the adherent cell fraction was harvested and assayed for SARS-CoV viral titer. Results clearly showed that even at high dilution, convalescent sera inhibited SARS viral replication (data not shown), presumably by blocking viral entry. This supports other reports indicating that SARS-CoV is not endocytosed through antibody mediated mechanisms and confirms a protective role for antibodies elicited either by the infection or through immunization [Traggiai et al., 2004; Shi et al., 2004]." on page 9, lines 4 to 11 as recommended.

3. "On the other hand, the ACE2 receptor has been described as functional SARS-CoV receptor. Maybe it
would be more interesting to investigate the expression of ACE2 in the PBMCs of donors who are showing different growth kinetics."

Ans: We agree with this point but ACE2 is not represented in the array used for this study. A future version of the array will address this.

4. "Gene expression analysis results should be confirmed by PCR for a subset of genes which were found to be differentially expressed. Furthermore it would be interesting if the observed changes are dependent of viral replication by use of UV-inactivated SARS-CoV."

Ans: We agree and further work will be performed to address all these issues. The sentence "In future developments of the model, it be interesting to compare the host response to different SARS-CoV isolates with inactivated preparations of the virus" has been added on page 11, line 25 to page 12, line 2 explain our view on comparing live and inactivated virus.

Minor Essential Revisions:

5. Labels on figures to be corrected.
Ans: Checked and corrected.

6. Abstract/Background: It should be clarified what the authors mean by "early immune response of SARS-CoV in PBMCs".
Ans: The sentence has been changed to "In this study, we therefore examine the immune response of SARS-CoV in human PBMCs over the first 24 hours" on page 2, line 6.

7. Legend for figure 1c
Ans: Corrected.

Reviewer 2:

Major Compulsory Revisions:

1. "The neutralization work presented in this paper is irrelevant to the gene expression profiling study. It should be deleted."
Ans: Figure deleted and text rewritten as mentioned above.

2. "SARS-CoV is a RNA virus. However, TLR9 binds to CpG DNA. The reviewer can accept the fact that TLR9 expression could be indirectly affected by SARS-CoV infection. However, unless the author could produce direct evidence to support their claim, the reviewer would not believe the CpG sequence of SARS-CoV could directly induce the TLR9 expression."
Ans: We agree that the CpG sequence may not be stimulating TLR9 directly, and the text has been amended. The sentence "TLR9 is known to respond to CpG signalling motifs (GTCGTT) and one possibility is that the virus is activating directly through this mechanism", on page 11, lines 13 to 15 has been changed and the sentence "An alternative explanation is that TLR9 is being stimulated by mechanisms unrelated to CpG recognition", on page 11, lines 20 to 21 has been added to address this point.

Thank you so much in advance and we look forward to hearing from you soon.

Warmest regards,

Lisa F. P. Ng,
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Ee-Chee Ren