Title: Cynomolgus monkeys (Macaca fascicularis) inoculated with Brazilian and Dutch swine HEV strains are successfully infected and exhibit hematological changes

Version: 2 Date: 5 August 2013

Reviewer: Jose M Echevarria

Reviewer’s report:

Major compulsory revisions

1. The Results section is too complex and long, and must be shortened by no less than one-half for the reader’s better understanding (I got lost in the jungle of data). Detailed description of results given in Table 2 and Figure 1 should be replaced by an overview of the data (i.e., frequency and timing of virus shedding and serum anti-HEV response, frequency of biochemical and liver alterations, etc.) and by a comparison of the findings among subjects inoculated with swine and human HEV strains. Adding individual data in regard to lymphopenia and monocytosis to Table 1 is also recommended.

2. Though HEV can be transmitted intravenously to humans by transfusion of plasma or blood derivatives, it would be merely accidental in comparison with the ingestion of contaminated water or food. Therefore, the haematological findings described by the authors might be an artifact from the route of inoculation chosen for the experimental infection that perhaps does not happen during the natural infection. In addition, the ability of these swine and human HEV-3 strains to overcome the species barrier and infect cynomolgus macaques might also be different after ingestion than after intravenous inoculation. These limitations of the study must be recognized in the Discussion section.

3. Two of the eight subjects of the experiment (F3 and J3) did not display detectable virus shedding during the study. For subject F3, the anti-HEV secondary-type response observed suggests an unnoticed, pre-existing immune memory against HEV that would have controlled efficiently the experimental infection. However, the subject displayed biochemical alterations and focal inflammation areas in the liver tissue, which the authors thought due to an "immunomediated response". Do the authors suggest that reinfection by HEV in a subject with specific immunological memory can lead to liver damage? What means exactly the expression "immunomediated response" in regard to such damage?

4. For subject J3, even the anti-HEV response was limited to IgG antibody, but, again, biochemical and histological alterations were observed. The authors propose either virus neutralization (by antibody present in the inoculum) or virus inactivation (due to a long storage time) to explain the findings. Though the rising of anti-HEV IgG would not be totally incompatible with these explanations, how to explain the liver damage if the inoculum contained just neutralized or inactivated
HEV particles?

5. There are similarities between the pattern of experimental infection recorded in subject I3 and the observations from persistent infections by HEV-3 among immunocompromised patients. However, the lack of biochemical alterations in this macaque on follow-up is a difference worth to be commented.

Minor essential revisions

1. Experimental design. If “changes of general behavior -such as anorexia- and well-being were verified by the veterinary staff, daily”, a comment on the results from such verifications should be added to the Results section. Alternatively, the sentence can be removed.

2. ELISA tests. Was anti-HEV IgG tested by two different EIA tests (MP and DiaCheck) on all samples? Is the MP Biomedicals test designed to test samples from cynomolgus macaques? Was it modified by the authors? Please, clarify how the anti-HEV IgG results from Table 2 and Figure 1 were obtained.

3. Real-Time PCR. Authors should provide a reference for the full details of the method used for quantifying HEV RNA by R-T PCR, or provide in other case these details in the text of the manuscript.

Discretionary revisions

1. I suggest replacing references 3-5 in the first paragraph of Introduction by “Journal of Medical Virology 2013, 85:1037-1045”, since it offers a full updated review of cases of acute hepatitis E reported from Latin America.

Level of interest: An article of importance in its field

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

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

The reviewer declares that no competing interests exist in regard to the content of the manuscript reviewed.