Reviewer's report

Title: Inducible nitric oxide synthase (iNOS) is expressed in Dengue-infected patients during acute phase and monocytes infected in vitro

Version: 1 Date: 27 November 2004

Reviewer: Douglas Perkins

Reviewer's report:

General

This manuscript presents interesting and important information about detection of iNOS in human mononuclear cells. There are actually only several reports illustrating that human mononuclear cells from healthy individuals generate NO production following treatment with cytokines, pathogens, and/or pathogen-derived products. It would be useful to add a brief paragraph in the discussion that illustrates this important fact.

After making some of the suggested changes that will strengthen the clarity of the manuscript, this work will be a nice addition to the literature.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1) Presentation of the data in the results section is somewhat confusing and unorganized. The reviewer suggests the following changes for the results section.

A. In the first Characterization Section of the results, more detail about the experiments would be useful.

B. The section immediately following the characterization which combines the in vitro and ex vivo experiments would be stronger and clearer if each of these topics were presented separately in discrete sections. For example, presenting the patient data first and combining the in vitro results will flow better for the readers and be more clearly defined.

C. For the in vitro experiments, there was an increase in iNOS in the presence of inactivated virus. This needs to be addressed in the discussion. It is interesting that there may potentially be virally associated products that can increase NO production, even though it is less than that of live virus.

D. On page 7 of the results it is stated that, "but did induce significant expression within monocytes (Figure 2B)." There are no statistics presented so it cannot be stated that these results are significant.

E. It is completely unclear what is going on with the experiments related to the first and second samples. This must be clarified in the results section.

F. In the last section on C6/C36 cells, it must be expanded and clarified to address the full range of the experiments. For example, until reading the figure legend, it was unclear what was being performed in this series of experiments. After reading it and spending large quantities of time trying to decipher these results – I am still not certain of the experimental design.
2) The following issue should be explained in more detail in the discussion. Why were NO donors added to the insect cells instead of the dengue-infected monocytes – or where they? According to the results section, monocytes were not used – according to the figure legend, monocytes were measured. This needs to be clarified further. Once it is clarified, it will require some slight discussion about whether or not the levels of NO generated from the donors actually target the virus, or if they are decreasing detectable virus because they are inducing apoptosis in the target cells contained the virus.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1) The statistics performed in the manuscript should be slightly expanded. The reviewer suggests the following:

A. It needs to be clarified whether a one- or two-sided t test was used.

B. Statistics for the different populations in Figures 4 and 5 would be useful. It will be important to determine if the data is normally distributed prior to performing the parametric or nonparametric measures.

C. Statistical comparisons in Figure 6 would be useful.

2) The figures are presented well be a few minor changes need to be addressed.

A. When describing Figure 4, it is stated in the figure legend that the line represents the average for each individual – I think you mean the population of individuals in each of the discrete categories.

B. In Figure 6, there are no error bars for the Day 6 controls. These should be included. Also, the upward error bar is not viewable in panel B. The y-axis, therefore needs to be increased.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

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

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.