Author’s response to reviews

Title: Acute dengue virus 2 infection in Gabonese patients is associated with an early innate immune response, including strong interferon alpha production

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Author’s response to reviews: see over
Dear Sir,

Please find enclosed our revised manuscript entitled *Acute dengue virus 2 infection in Gabonese patients is associated with an early innate immune response, including strong interferon alpha production.*

**Response to reviewer Daniel Libraty (referee n°2)**

**Major compulsory revisions**

1. **Major- overall, authors have removed some of the previously overstated conclusions. The paper would be further improved by discussing in a little more detail that serum IFN-alpha was measurable up to 5 days after illness onset in some of their patients.**

   We have improved our discussion of this point:

   “Interestingly, in another study, IFN-α levels fell after the peak in viremia, one day before defervescence in Thai children aged 6 months to 14 years with secondary DENV-3 infection [46]. In our study, more than 85% of DENV-2 infected patients (data not shown) still had fever 5 days after symptom onset, and high IFN-α levels persisted throughout the acute phase. IFN-α levels could not be measured on the day of defervescence.”

2. **In data, show numbers of subjects at each time point.**

   All the figures have been modified as requested.

3. **In discussion, the IFNalpha data is something that can be better compared/contrasted with other studies in the literature from Asia or Americas, and fits into their final conclusions.**

   We have improved the first paragraph of the discussion, and included new references:
"This is the first study of the innate immune response to DENV in an African population. One of the most significant results of this study is the sustained elevated levels of IFN-α2 throughout the acute phase of the illness in all DENV-2-infected patients. IFN-α, mainly produced by dendritic cells and macrophages [42], is a first line of host defense, limiting viral replication. In addition to its antiviral affects, IFN-α induces the expression of major histocompatibility complex class I molecules, enhancing antigen presentation and thereby triggering the acquired immune response. IFN also induces antigen-specific CD8+ cell responses and chemokine production, leading to activation of lymphocytes and monocytes and their recruitment to sites of inflammation [43]. A previous study of Thai children aged 5-14 years with DF showed elevated IFN-α levels only on D1 and D3 after fever onset [13]. This slight discrepancy with our findings might be due to immunological immaturity, detection of different IFN-α subtypes [44], infection by different DENV serotypes, assay sensitivity and specificity, or differences in the choice of standards. Our results confirm, in DENV-2-infected adults, the potential role of IFN-α in DENV infection, as shown in vitro and in animal studies [9, 10, 12]. Studies exploring the relationship between viral load and the clinical severity of dengue infection have given conflicting results [8, 45-47]. We found no correlation between virus load (measured by qRT-PCR) and IFN-α levels ($r^2<0.4$; data not shown). This could be explained by the absence of severe cases of DENV infection, the consistently high IFN-α levels in all DENV-2 infected patients, the small size of the study population, and infection by a different DENV serotype. A more recent study in Taiwan suggested that patients with secondary DEN-2 infections mount an altered immune response with lower IFN-α levels, 3-7 days after symptom onset, associated with the onset of DHF [48]. This suggests that the earlier proinflammatory reaction associated with IFN-α may be critical for the pathogenesis of secondary DENV-2 infections."

4. Statistics - the authors explanation for not incorporating a multiple comparison statistical test is difficult to understand, and I believe not correct

To address the reviewer’s concern, we reduced the Type I risk from 0.05 to 0.01, applying a Bonferroni correction for each group of cytokines (growth factors, pro-inflammatory and anti-inflammatory factors, chemokines, and cytokines associated with adaptive responses).

The Statistical Analysis section has been modified:
“Student’s t test was used to compare normally distributed cytokine concentrations between the patients and controls, while the Mann Whitney Wilcoxon test was used for non normally distributed data. Normality was tested with the Shapiro-Wilk test. P values below 0.01 were considered to indicate significant differences (to take account of multtesting for the groups of cytokines, the type I risk was reduced from 0.05 to 0.01 using Bonferroni correction). STATA 10.0 (College Station, Texas USA) and SavGIS 9.05 software (IRD, France) was used for all analyses.”

Minor essential revisions:

1. **Authors still refer to kinetic analysis- they should remove this characterization, as they did not measure longitudinal changes in cytokine levels.**

Corrected.

2. **In discussion, sentence “Decreased IFN-alpha levels have been shown after the peak in viremia...” makes it sound like IFNalpha levels were low. Sentence should likely state that IFN-alpha levels were decreasing after the peak in viremia...?**

We have modified this sentence:

“Interestingly, in another study, IFN-α levels fell after the peak in viremia, one day before defervescence in Thai children aged 6 months to 14 years with secondary DENV-3 infection [46].”

Furthermore, virus load in plasma was measured by real-time quantitative PCR with a quantified synthetic DENV transcript kindly provided by University of the Mediterranean (Prof. X. De Lamballerie), which was used as a standard for quantifying DENV-2 RNA. We have modified the “Materials and Methods” and “Results” sections to include this new information.
Materials and Methods:
“A quantified synthetic DENV RNA transcript kindly provided by the University of the Mediterranean (Prof. X. De Lamballerie) was used as standard for the quantification of DENV-2 RNA in positive samples. All amplifications were performed in duplicate.”

Results:
“**DENV-2 viral load**
Viral load (VL) was measured in plasma by real-time quantitative PCR in DENV-2-infected patients. Mean VL was $1.2 \times 10^6 \pm 4.4 \times 10^1$ cDNA copies/mL ($2.7 \times 10^4$ to $5.1 \times 10^7$ cDNA copies/mL). VL did not vary significantly with age, sex or the day of sampling after symptom onset (data not shown).”

We have also added a new reference to the introduction:
“Interestingly, DENV-2-infected monocyte-derived dendritic cells in vitro fail to prime T cells, due to the lack of IFN-α/β produced in those cells after infection [11].”

The manuscript has been checked for English usage by a professional scientific editor.