Reviewer's report

Title: Excessive proinflammatory cytokine and chemokine responses of human monocyte-derived macrophages to enterovirus 71 infection

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Reviewer: Satoshi Koike

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Excessive proinflammatory cytokine and chemokine responses of human monocyte-derived macrophages to enterovirus 71 infection by Gong et al.

General comments

Proinflammatory cytokines and chemokines are thought to play roles in pathogenesis of severe neurological disease caused by EV71. However, the cellular sources of these cytokines and chemokines are not known. The authors hypothesized that the macrophages are one of the candidate. The authors, therefore, examined the susceptibility of monocyte-derived macrophages to EV71 infection and induction of cytokine and chemokine upon infection. They also examined the expression levels of genes for viral RNA sensors. They concluded that the monocyte-derived macrophages are one of the target cells of EV71 infection and they may play an important role in the pathogenesis through production of a large amount of proinflammatory cytokines and chemokines.

I have several concerns in describing the experimental results and interpretation of the results in the following.

Major compulsory revisions

1. Fig. 1 and the first paragraph in the result section. This reviewer did not think that the authors described the EV71-infected macrophages sufficiently. Did macrophages show cytopathic effect or not? Did they survive 24h post-infection? In Fig1C, not all the cells looked being infected by EV71 even though EV71 was infected at MOI of 5. Were all cells infected if the authors employed a higher MOI? In D, the authors showed kinetics of viral multiplication using realtime PCR. Did macrophages produce infectious progeny particles or not? The increase in the amount of viral genome was only three-fold. If the viral genome increased only such small amount, I wonder it can be detected by IFA. Is it possible to conclude “Effective infection…” written in the headline of the paragraph?

2. In Fig. 2, the authors presented data for production of cytokines after EV71 infection. They claimed that induction of IL-1b, IL-6 and TNF-a was observed. However, this reviewer thinks that the levels of the cytokines were very small and it would be very difficult to discuss the difference between the cytokine levels of infected and mock-infected samples. Even if they were certainly different, this reviewer was not convinced that these small amounts of cytokines are effective in vivo and that “excessive proinflammatory cytokine and …” in the title of the
manuscript is adequate.

3. In page 9, lines 5-6, the authors discussed that viral proteins of EV71 may be recognized by TLR-2. This is not logically correct. Even though TLR2 expression is elevated after EV71 infection, this does not mean that the EV71 is recognized by TLR2.

**Level of interest:** An article of limited interest

**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:**

I declare that there is no competing interests.