Review of: "SARS-CoV-2 accessory protein ORF8 decreases antibody-dependent cellular cytotoxicity"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

In this study, the authors evaluated the effect of SARS CoV-2 protein ORF8 on PBMC. They found that ORF8 binds CD16 and downregulates the level of CD16 at the surface of monocytes and NK cells, reducing the ability of these cells to perform ADCC. Monocyte-mediated ADCC was predominantly affected ORF8 binding. This is an interesting study which could reveal a new viral immune escape machanism. However, the following specific points should be addressed.

1. All ORF8 experiments were performed with or without ORF8 and did not include a control protein. I think it is important to add at least one protein as negative control (produced under similar experimental conditions of ORF8) to assess specificity of binding. Moreover, a negative control protein should be included in CD16 downregulation and ADCC experiments.

2. ADCC experiments were performed with plasma from convalescent, vaccinated or convalescent and vaccinated individuals. Control plasma must be added. Moreover, some ADCC experiments should be performed using a well-characterized monoclonal antibody approved for clinical use, i.e. Rituximab, Cetuximab..., with an appropriate ligand-expressing target cell line.

3. Was there a correlation between CD16 expression and ADCC?

4. Representative flow cytometry dot plots should be shown for figures 1C, D and figure 2 B C D E

5. On page 5 the authors state: "*As shown in supplemental figure 1A, addition of soluble ORF8 to purified monocytes modulated CD16 levels in a manner similar to when added to total PBMCs, therefore suggesting a direct effect of ORF8 on monocytes. To verify if the decreased CD16 levels at the surface of NK cells also depends on the presence of monocytes, ORF8 was added to monocyte-depleted PBMCs. As shown in supplemental figure 1B, the small decrease on NK cells was not observed upon monocyte depletion, confirming the role of monocytes in ORF8-mediated downmodulation of CD16 on NK cells.* However, in order to confirm such statements, the number of experiments should be increased, as only two samples were tested.