Reviewer's report

Title: Modulation of natural killer cell-activity directed against ovarian cancer cell lines by the 38 kDa-antigen of mycobacterium tuberculosis

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Reviewer: Hiroyoshi Nishikawa

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The manuscript by Gottschalk N. et al. describes the augmentation of natural killer (NK) cell function by PstS-1, the 38 kDa-preparation of M. tuberculosis. The prognosis of ovarian cancer is still poor, and long-term survivors are limited. The authors addressed this issue and explored the potential of PstS-1 as an enhancer of anti-tumor immune responses. They examined activation status, cytokine release and cytotoxicity of NK cells after stimulation with monocytes and PstS-1. While PstS-1 did not have any direct stimulatory capacity to NK cells, stimulation of NK cells with monocytes and PstS-1 induced upregulation of CD69 and enhanced production of IFN-g, IL-15 and IL-18 compared with monocytes alone. However, cytotoxic and ADCC activity was not influenced by the addition of PstS-1.

The most important point that this study made is to show the augmented NK cell function, such as cytokine release by stimulation with PstS-1. However, addition of PstS-1 did not further enhance the killing capacity of NK cells. Given the major function of NK cells in anti-tumor immunity is direct killing against tumor cells, this manuscript needs changes to clarify the role of enhanced cytokine secretion by NK cells stimulating with PstS-1.

Major comment;

This study lacks critical experiments addressing the role of enhanced cytokine secretion by NK cells after stimulation with PstS-1 and monocytes compared with monocytes alone. The authors proposed the augmentation of T-cell function by the enhanced cytokines, but they do not show any experimental data. In addition, while they claim some clinical applications, they do not observe any enhanced tumor lytic activity by this strategy. As IFN-g may induce the MHC class I expression, it may reduce NK cell killing capacity.

Specific points;

1) In Fig. 3, expression of an activation marker CD69 was analyzed. It seems that both % of activated cells and CD69 expression was enhanced. It should be helpful to show the % positive cells and MFI of CD69 staining.

2) In Fig.4, the authors addressed cytokine secretion using real-time PCR. As mRNA expression sometimes does not reflect protein doses, cytokine assays at protein level (such as ELISA) should be included. In addition, while IL-15, IL-18 and IFN-g secretion was augmented, it is necessary to examine which cytokine
(s) is critical for the augmented NK activity by blocking assay.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I declare that I have no competing interests.