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: Junko Matsuzaki

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The authors investigated whether PstS-1 which is known as an immunogenic antigen from M. tuberculosis could directly or indirectly activate NK cells derived from healthy donor PBMC. It was found that PstS-1 did not show any direct effect on NK cells. However, when NK cells were cocultured with monocytes, PstS-1 upregulated CD69 expression and cytokine production. Enhancement of ADCC to EGFR was observed on EGFR positive but not negative tumor target. The weakness of the study was a lack of new findings. Most of observations in this manuscript were previously reported by others.

Major Compulsory Revisions

1. Because there was no evidence that PstS-1 directly modified NK cell function, the title and several descriptions in the results are overstated for its effect on NK cells.

2. PstS-1 has been reported to be recognized by T cells and to stimulate innate immune cells through TLR-2 and TLR-4. According to previous reports, NK cell does not express these TLRs nor recognize antigens that are recognized by T cells. It is recommended to describe why the authors attempted to test the direct effect of this reagent on NK cells.

3. PstS-1 appeared to enhance NK cell activation through monocyte activation. Data showing cytokine production from monocytes+PstS-1 in the absence of NK cells would be important in Figure 4 because IL-18, IL-15 and IL-12 are DC-derived cytokines as authors discussed whereas IFN-g is dominantly produced by NK cells.

4. Authors concluded that PstS-1 contributed to full-activation of NK cells though it is not clear what the criterion was used for full-activation. Authors examined CD69 expression to evaluate NK cell activation. To study targeting of MIC-expressing ovarian tumors, analyses of NKG2D expression would be more important.

Discretionary Revisions

1. Authors demonstrated that IFN-g production but not cytolytic function was enhanced by PstS-1 treatment. Because there are two distinct NK cell subsets (IFN-g producing CD56brightCD16- and cytolytic CD56dimCD16+) in human PBMC, investigating whether PstS-1 could activate CD56brightCD16- subset
selectively would be interesting.

2. Authors use the same histogram data on Fig 1a/Fig 3a and Fig 1b/Fig 3b. Figure 1 and Figure 3 should be combined.

3. IFN-g ELISA and CD69 expression were shown by two different ways; fold change in some figures, % or concentration in other figures. It would be better to show the data using the same unit.

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

**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.