Author’s response to reviews

Title: Functional capacity of Natural Killer cells in HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients

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Author’s response to reviews:

Dr. Rudra Channappanavar
Editor BMC Infectious Diseases
Salvador, 03 December 2018.

Dear Dr. Channappanavar,

We would like to thank you for the opportunity to revise our paper for resubmission. We would like to express our appreciation for the relevant comments and criticism from the reviewers. It is our opinion that their contributions have resulted in an improved manuscript and we have responded to the reviewers’ suggestions as follows:

Technical Comments:
Query 1) Please add all the co-authors email address in the title page of your paper.
Reply: We have added the email addresses as requested (lines: 3 to 8)
Query 2) Please add heading for the Declarations sections and format as seen below:

Declarations
- Ethics approval and consent to participate
- Consent to publish
- Availability of data and materials
- Competing interests
- Funding
- Authors’ Contributions
- Acknowledgements
- Authors’ Information

Reply: We have included a Declaration section in the manuscript (lines 268-280).

Query 3) Please add the ethics and consent to participate statement (mentioned in the Methods section) to the Ethics and consent to participate section of Declarations.

Reply: We have added the ethics and consent to participate statement in the declaration section (lines 270-273).

Query 4) Please include a statement on consent to participate in the “Ethics approval and consent to participate” section of the Declarations, and state whether consent was written or verbal, together with an explanation if it was verbal.

Reply: All participants signed a term of informed consent. We have included this information in the Declaration section of the manuscript (lines 270-273).

Query 5) Availability of Data and Materials - Please include an “Availability of data and materials” statement section in their article detailing where the data supporting their findings can be found. BioMed Central strongly encourages all datasets on which the conclusions of the manuscript rely to be either deposited in publicly available repositories (where available and
appropriate) or presented in the main paper or additional supporting files, in machine-readable format (such as spreadsheets rather than PDFs) whenever possible. However, if you do not wish to share your data, please state that data will not be shared, and state the reason.

Availability of data and materials: http://www.biomedcentral.com/submissions/editorial-policies#availability+of+data+and+materials

Availability of data and materials statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

• The datasets generated and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]

• The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

• All data generated or analysed during this study are included in this published article [and its supplementary information files].

• The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

• Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

• The data that support the findings of this study are available from [third party name] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [third party name].

• Not applicable. If your manuscript does not contain any data, please state 'Not applicable' in this section.

Reply: We have included the following statement: The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request (Lines 278-280.)

Query 6) Figure Legends - No title or legend should be attached to the figure image. Instead, could you please include a Figure Legends section to appear after the References section? Also, please remove the captions from the files.
Reply: We have excluded the legends attached to the figures, and have included a Figure legends section (pages 19-20, lines 420-460.)

Reviewer #1

It is quite obvious that a lot more work has to be done to conclusively show that this is causal than just correlative. While the reviewer do appreciate the how difficult it would be to do such studies with human samples, it would add to the story if the authors could further alter the level of NKp30 using siRNA mediated knockdown or lentiviral overexpression strategies to further ask if it changes the cytotoxic capability of NK cells.

Also in the discussion the authors have touched upon the various isoforms of NKp30 including one with immunosuppressive functions. Given the fact that qRT-PCR remains an easy technique, it would be an additional piece of data if the authors could manage to quantify the relative expression of various isoforms expressed by NK in the groups they are studying. This might shed light into why in spite of higher basal expression of cytotoxic markers they fail to upregulate them upon stimulation.

Reply: The reviewer raises a very interesting point, in that the cytotoxic capability of NK cells should be modulated by altering the level of NKp30 in cultures. In an attempt to address this issue, we have performed a cytotoxicity assay using K562 target cell stimulation, in the presence or absence of an anti-NKp30 monoclonal antibody, to block its receptor. Interestingly, blockage of the Nkp30 receptor decreased cytotoxic activity (CD107a) and IFN-γ expression only in asymptomatic HTLV-1-infected individuals. We have included this information in the abstract (lines 31-32; 40-41), methods (lines 118-119), results (lines 175-180; Figure 5) and discussion (lines 194-197) sections.

Minor comments:

Query 1) Line 60: Please correct grammar "It has been reported a lower frequency of circulating NK cells in 61 patients with HAM/TSP compared with asymptomatic carriers (16-18)."

Reply: We apologize for this oversight and have rephrased sentence: “A lower frequency of circulating NK cells has been reported in patients with HAM/TSP in comparison to asymptomatic carriers” (lines 71-72).

Query 2) Line 71: Please correct grammar and sentence structure " to diagnose HAM/TSP(19). Were included twenty-two individuals with HAM/TSP. Twenty"
Reply: We have corrected this sentence as follows: “Asymptomatic individuals (AS) were included if neurological examinations were normal and no clinical complaints were reported. A total of 29 individuals with HAM/TSP and 30 asymptomatic carriers were included. Eighteen laboratory staff volunteers and healthy blood donors served as non-infected controls.” (lines 82-86)

Query 3) It will be ideal to include either isotype controls or FMO (fluorescence minus one) for the markers that are being used for phenotypic characterization of NK cells from subjects to be able to more accurately quantify their relative expression (Fig 1 and 2).

Reply: Isotype controls were used for each antibody, as per manufacturer recommendations. The following list of isotype controls was used in the immunophenotyping assays: (APCCY7-IgG2a); (PECY7-IgG1); (BV421-IgG1); (PE-IgG1-extracellular); (PE-IgG1-intracellular); (FITC-IgG1); (BV510-IgG1); (AF647-IgG1) (lines 100-102.) We have modified Figure 1 and included histograms for each of the isotype controls with overlays of the studied markers.

Query 4) Representative flow plots for cytokine staining as well as cytotoxic molecule staining might come handy to appreciate the strength of the signal (Fig 3).

Reply: As requested, we have removed the representative flow plots from Figure 1 and included them in a new Figure 4 to illustrate the strength of the signal.

Reviewer #2:
Query 1) providing absolute number and dynamics of NK cells in donors, especially HAM/TSP patients;

Reply: Unfortunately, we are unable to provide the absolute number and dynamics of NK cells for the individuals included in this study.

Query 2) comparing direct cytotoxicity (death of target cells, such as K562) in addition to NK cell markers;

Reply: In an attempt to compare direct cytotoxicity, we performed a cytotoxicity assay using K562 target cell stimulation, in the presence or absence of anti-NKp30 monoclonal antibody to block the NKp30 receptor. Interestingly, blockage of the Nkp30 receptor decreased cytotoxic activity (CD107a) and IFN-γ expression exclusively in asymptomatic HTLV-1-infected
individuals. This information has been included in the abstract (lines 31-32; 40-41), methods (lines 118-119), results (lines 175-180; Figure 5) and discussion (lines 194-197) sections.

Query 3) demonstrating the correlation of NK cell characteristics with virus load and other immune components (such as CD4 and CD8 number, phenotypes).

Reply: We were unable to find any correlations between proviral load and molecules expressed by CD4 and CD8-T cells. The absence of a correlation between HTLV-1 proviral load and the evaluated markers may likely be due to the relatively small number of individuals evaluated. However, while our data do not provide evidence that proviral load is associated with another NK marker (not tested in this study), we can highlight that NKp30 expression was, for the first time, found to discriminate asymptomatic individuals from HAM/TSP patients. We have included this issue in the discussion section (lines 234-238.)

Query 4) a table containing information of donors (age, gender, hospitality, et al) might be helpful;

Reply: We have included a table (Table 1) that details this information of the donors.

Query 5) flow plots of NK cell characteristics from all three groups (HAM/TSP, AS, CTR) need to be included in the manuscript.

Reply: We have added representative flow plots for each of the three groups in Figures 1 & 3.

Sincerely,

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