Reviewer’s report

Title: Cytotoxic response persists in subjects treated for tuberculosis decades ago

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Reviewer: Thomas Scriba

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Savolainen et al have revised their paper and addressed most of the concerns raised in the previous round of review. However, a number of technical issues remain a source of concern. There are issues with interpretation of the data and it is not clear how the results present an advance in the field of TB immunology or biomarkers for diagnosing TB. I have listed some of my concerns.

1. Abstract: The first line of the abstract introduces the importance of accurate biomarkers for diagnosing TB. However, the paper does not present results that lend themselves to the discovery of such biomarkers. As such, the abstract misleads the reader. It may be more appropriate to state that the study aimed to identify functional differences in Mtb-specific immune responses in the different groups. Further, seemingly important aspects of the study design, especially those relating to the different clinical groups, are absent from the abstract. This does not allow the reader to assess what the question is, how the study was designed or carried out, or how to interpret the results. The abstract refers to “different stages of TB infection and in persons treated for TB decades ago” in the methods section and in the results section only mentions that no differences were observed between treated TB patients and “other groups”. If I were to come across this abstract on Pubmed I would not be able to draw any meaningful information from it. I suggest the authors revise the abstract carefully to clearly state the scientific question being addressed (also in the intro of the main text), bring out the main results and messages and then, if appropriate, draw clear and meaningful conclusions.

2. The authors show “cumulative” frequencies of GrB and IFN-#-producing cells in Figure 2 and report a difference in these cumulative frequencies between patients treated with the 3-drug modern therapy and “the other groups”. What does cumulative mean? Is this the sum of the frequencies of GrB and IFN-#-producing cells? If so, I would argue that this approach may artificially inflate the inter-group differences. It is likely that at least some cells co-express GrB and IFN-#. Adding these frequencies together may therefore count some of the same cells twice, which artificially inflates any difference between the groups. The fact that no differences were observed between these groups when GrB and IFN-#-expressing cells and IFN-#-expressing cells were analysed separately suggests that this may well be the case.

3. The sample sizes in the different analyses shown in Figures 1 and 2 are different. For example, in figure 1 the “mod” group shows 7 datapoints for GrB
expressing cells and 5 for IFN-# expressing cells. What is the reason behind this? This should be stated in the manuscript. These sample sizes of 5-7 for some groups are also very small – likely too small to perform robust statistical tests and draw meaning conclusions about differences in specific immune responses, which are notoriously heterogenous.

4. What is the difference between Fig 4B and C? It is not explained in the figure legend and also is not clear from the results section.

5. The authors have now included a new paragraph on page 9 (lines 250-257) of the marked-up version about correlations between the frequencies of different functional subsets of T cells. These data should be included in the figures.

6. The discussion about the differences in “cumulative” immune responses between the groups is speculative and should be toned down. For example, the statement that “the 3-drug therapy elicits better protection in terms of higher immunological alert by maintaining higher numbers of potential CTLs” is not supported by data and highly speculative. Epidemiological data clearly show that persons with previous TB, even if successfully treated, have a significantly elevated risk of disease when re-exposed. What do the authors means when referring to “better protection”? It is also not clear what “higher immunological alert” means.

7. The discussion of the memory phenotype results should be revised to be more clear and convey a clear message. It should take into account differences in CD4 and CD8 T cells when discussing the results from this study and also those in the published literature. Phenotypes and functions of CD4 and CD8 T cells are typically highly distinct. For example, CD45RA+CCR7- CD4 T cells are generally a very minor subset in the published literature, while this subset is more frequent in the CD8 compartment. What does the following statement mean: “the Tcm pool had the highest proportion of antigen-specific effector cells”? 

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

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests