Reviewer’s report

Title: SBF-1 inhibits contact hypersensitivity in mice through down-regulation of T-cell-mediated responses

Version: 0 Date: 24 May 2019

Reviewer: Cheryl Rockwell

Reviewer's report:

This manuscript describes the effect of a saponin analog, SBF-1, on T cell response to polyclonal activators in vitro and TNCB (picryl chloride) in an in vivo model of contact hypersensitivity. The authors demonstrate that SBF-1 inhibits proliferation, expression of activation markers, cytokine expression and induce apoptosis in activated T cells in vitro. They also show that SBF-1 reduces inflammation and swelling following challenge in the in vivo model of contact hypersensitivity. Although this study is interesting and of potential relevance therapeutically, it would be helpful if the authors could provide a more detailed statement concerning the rationale for initiating this experiment. In addition, the following points should be considered:

1. The manuscript needs a bit more editing by a native English speaker for grammar, syntax and stylistic issues.
2. Since the authors are studying contact hypersensitivity rather than autoimmunity, it seems like it would be better if the first sentence of the abstract related to CH instead of autoimmunity.
3. When the authors use the term "naïve" T cells, did they intend to say that the T cells are unactivated or do they mean that naïve T cells were isolated from lymph nodes either magnetically or by cell sorting. If they mean the cells were not treated with activator, then it would be more accurate to call them "unactivated". If naïve T cells (CD62L-hi CD44-lo) cells were isolated, this should be added to the methods section.
4. It is important to note that the MTT assay does not specifically measure either proliferation or viability, but rather mitochondrial metabolic activity. In this study where cells are both actively dividing and dying at the same time, the MTT assay cannot be used to distinguish between decrease in proliferation or increase in cell death. The text should be clarified to indicate that the MTT data indicate that SBF-1 reduced mitochondrial metabolic activity of activated, but not resting, T cells.
5. For the group labeled "Model" in the figures (such as in panel 4D), is this panel actually a VH control (as described in the Methods section)? If so, this group should be labeled VH (vehicle) or some equivalent.
6. The figure referenced on line 26 of p. 10 should be 4a rather than 1a.
7. Can the authors describe why they chose to switch vehicles from sensitization to challenge?
8. Can the authors provide more information about the rationale for this study?
9. Can the authors please provide more detailed information in the Methods section? Specifically,
   a. Where did the SBF-1 come from?
   b. Is the CD2 a typo in the Reagents section? Did you intend to write CD3?
   c. How was T cell purity quantified?
   d. PCR analysis needs to be added to the Methods section
   e. Please provide information about how the histopathology scoring was conducted and what the scale means with respect to injury/severity
   f. Can the authors clarify whether they performed both an ANOVA and Student's t test for the statistical analysis, and if so, why? Or did the authors run an ANOVA followed by a post-hoc test, as is
more typical

g. Please include the method used for the tritiated-thymidine uptake assay.
h. Please include the method used for MTT assay.

10. 72 h seems like it might be past the peak of CD69 and CD25 expression. Does it make sense to look at an earlier time point like 24 or 48 h?

11. In order to support the statement that SBF-1 is less immunosuppressive than CsA or Dex with respect to effects on unactivated T cells, the effect of SBF-1 on the viability of unactivated T cells should be assessed by Ann V/PI analysis and CsA should be included for reference.

12. Although the data with p-Akt points to a possible mechanism, this has not been conclusively shown with an inhibitor or other similar approach. The authors should modify the language a bit to indicate that p-Akt is a possible mechanism.

13. Please plot the body weight as percent change in body weights—this will make it easier to see if either of the compounds caused any modest changes in body weight.

14. The figures are nicely formatted and easy to interpret—very nice!

Minor Comments

1. It might be worth mentioning that picryl chloride is the same thing as TNCB as many investigators are familiar with TNCB.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:
1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license ([http://creativecommons.org/licenses/by/4.0/](http://creativecommons.org/licenses/by/4.0/)). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal