Author's response to reviews

Title: Immunomodulation of murine collagen-induced arthritis by N, N-dimethylglycine and a preparation of Perna canaliculus

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Author’s response to reviews: see over
Dear Ms. Emma Parkin,

The authors appreciate your consideration and the helpful comments of the Reviewers. We trust we have responded adequately to the Reviewers’ comments and hope that you now find the revised manuscript appropriate for publication. Please find our detailed point-by-point response below.

Sincerely,
Brian R. Lawson

Reviewer #1 (NM Davies)

*Minor Essential Revisions*

1. We agree with the reviewer and will confirm that all *in vitro’s* and *in vivo’s* are in italics throughout the manuscript.
2. We have changed “if” to “of” on line 16 of the abstract.
3. Was the neutrophil superoxide assay performed with DMG? No, we did not conduct this experiment.
4. As suggested, animals and treatment groups section has been moved before the section on CIA induction.
5. *The authors state in line 4: “DMG plus Perna (at the previously indicated concentrations).” Which are the concentrations, and where were they previously indicated?* We have now clarified this section.
6. *Authors should include a summary table similar to Table 1 to summarize the results in this section for enhanced clarity.* We have provided a new Table (Table 2) as suggested for clarity.

*Other comments/observations*

1. We agree with the reviewer that PD/PK studies are now highly warranted for therapeutic dosing calculations.

Reviewer #2 (R. Toes)

*Comments:*

1. “…the effects of these drugs on TNF-α levels in the sera could be analyzed.”
   a. We agree with the Reviewer on the importance of TNF-α in the pathogenesis of RA/CIA, but we no longer have the sera from the treated animals to analyze. This point is exactly why we performed the *in vitro* assays with monocytes (high level producers of TNF-α) depicted in Figs 1 and 2. After the CIA studies were completed, however, we treated lupus-prone MRL-*Fas*<sup>lpr</sup> mice for 6 months and tested their sera for levels of several cytokines, including TNF-α. Fully understanding that these results are preliminary and do not necessarily reflect the situation in the arthritic mice, we can report that treatment with Perna did reduce TNF-α levels in the treated MRL-*Fas*<sup>lpr</sup> sera (unpublished data).
2. “…Fig 1-3 may be eliminated because they are not closely related to the other data presented in the manuscript.....it is a bit confusing that the pro-inflammatory property of DMG on human monocytes (Fig 1) contrasts the in vivo anti-inflammatory effect on rat CIA.......rat/murine cell lines should be included.....more importantly, crucial controls are missing in these figures such as the exclusion of possible toxicity on cells, especially in the case of Perna (Fig 2 & 3).”
   a. We respectfully disagree with the Reviewer regarding Figs 1-3. Those Figs speak to the ability of the two compounds to modulate cytokine responses, in particular TNF-α, and we believe are necessary for interpretation of the *in vivo* disease results. We think associating TNF-α reductions, regardless in which cell type, with reduced CIA is logical. We were also surprised by DMG’s differing effects in the two different CIA models (rat versus mouse). The Reviewer is correct to point out what we suspect, that is species-variability.
   b. We agree with the Reviewer that in future studies, the role of species-specificity in regards to DMG activity should be addressed using several different cell lines from several different species, but we feel that is beyond the scope of the current work and would not add new information to the *in vivo* efficacy results.
   c. We have added to the “Perna and DMG” section in the Methods (page 5) text addressing this important issue. We agree with the Reviewer that these data should have been mentioned in the manuscript.

3. “…adding immunohistochemistry data demonstrating the structure and cell infiltration of the affected joints to table 1 and Fig 5 would be helpful.”
   a. We agree with the Reviewer in this regard, unfortunately those tissues are no longer available.

4. “…the percentage of T/B cells in the draining lymph nodes should be given as well?”
   a. We felt that analyzing splenic immune cell subsets was adequate, particularly in light of the very minimal changes observed. Functional assays were not conducted.