Author's response to reviews

Title: TRAIL Death Receptor-4, Decoy Receptor-1 and Decoy Receptor-2 expression on CD8+ T cells correlate with the disease severity in patients with rheumatoid arthritis

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Author's response to reviews: see over
July 6, 2010

Editor in Chief of the BMC Musculoskeletal Disorders Journal

Ref: MS 1172304023373206

Dear Editor:

I would like to submit the revised manuscript (2nd revision) entitled “TRAIL Death Receptor-4, Decoy Receptor-1 and Decoy Receptor-2 expression on CD8+ T cells correlate with the disease severity in patients with rheumatoid arthritis” for publication in the BMC Musculoskeletal Disorders journal. I have pasted below a response letter to reviewers’ comments.

The data in the manuscript is original and the manuscript is not under consideration elsewhere. All authors have read and approved the final version of the manuscript, its content, and its submission to the BMC Musculoskeletal Disorders. Authors declare that they have no conflict of interest.

I am looking forward to hearing from you. Sincerely,

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Revision 2

Title: TRAIL Death Receptor-4, Decoy Receptor-1 and Decoy Receptor-2 expression on CD8+ T cells correlate with the disease severity in patients with rheumatoid arthritis

We thank the reviewers for their insight and helpful suggestions. Below are the comments from the reviewers and our responses to them.

Reviewer 1

Comment 1: The most crucial issue is regarding the FACS data presented in Figs. 2 and 3. In my original critique, I brought up the issue of why the increase in TRAIL receptor expression was only observed in the CD8+ cells (Fig. 3), but not in the CD4- cells (Fig. 2, these cells should be CD8+ for the most part). If you examine the data in Fig. 2A and 3A, the RA patients were all elevated compared to normal control. This raises the question of why the original Fig. 2B did not show any increases in TRAIL receptor expression in the CD4- population. Why would the FACS results not matched that of the graph?

In response to my comment, the authors now show results from a different patient in Fig. 2B. Although the TRAIL receptor positive cells can be detected clearly in the new figure, the original FACS results still bother me since the authors did not offer any explanation on why the first set of results were negative. Were the FACS staining results in Fig. 2B and Fig. 3B from the same donors? If they are from the same donor, it should at least be internally consistent. In addition, the authors still have not shown the isotype staining controls despite claiming that they were done.

Action taken: Unfortunately this reviewer has been misled by our previously selected flow cytometry graph (Figures 2B and 3B). These graphs do belong different patients and not from the same patient. If we look at clearly to Figure 2A there are some RA patients whose CD4+ T cell associated TRAIL and TRAIL receptor expression levels were close to those of the control patients. This was what was originally reflected on our previous Figure 2B. Upon the suggestion of the reviewer and to prevent any misunderstandings, we replaced Figure 2B with another RA patient’s data showing elevated levels of TRAIL and TRAIL receptor expression on CD4+ cell population (mainly CD8+ T cells). This answers the most crucial issue brought up by the reviewer regarding the FACS data presented in Figs. 2 and 3.
which was why the increase in TRAIL receptor expression was only observed in the CD8+ cells (Fig. 3), but not in the CD4- cells (Fig. 2, these cells should be CD8+ for the most part).

Reviewer 1: Why would the FACS results not the authors show not matched that of the graph?
There are twenty data points belonging to twenty different RA patients in Figure 2A and 3A. We could only show one example from two separate patients out of twenty in Figures 2B and 3B.

Reviewer 1: Although the TRAIL receptor positive cells can be detected clearly in the new figure, the original FACS results still bother me since the authors did not offer any explanation on why the first set of results were negative. Were the FACS staining results in Fig. 2B and Fig. 3B from the same donors? If they are from the same donor, it should at least be internally consistent. In addition, the authors still have not shown the isotype staining controls despite claiming that they were done:
Flow cytometry staining results of Fig 2B and 3B are not from the same donor. No flow cytometry assays can be done and interpreted without internal controls. To satisfy this reviewer, we have pasted below isotype staining controls from flow cytometry assays just for the reviewing purposes.
Upper Panel: Unstained sample, Forward Scatter versus Side Scatter for grouping cells

Lower Left Panel: Isotype staining control for CD4 and CD8 staining

Lower Right Panel: Isotype staining control for TRAIL and TRAIL receptor staining

These staining processes were performed each time a control or a patient sample was analyzed.

Flow cytometric analysis was performed on an Epics™ XL™ (Beckman Coulter). The flow cytometer was calibrated and verified daily with Flow Check™ and Flow Set™ fluorospheres (Beckman Coulter).

Reviewer 2
Comment 1: The authors have clarified all the questions. They have answered the comments and add some points to the discussion section of the manuscript.

Response: We again thank to both reviewers for their useful comments, which obviously increased scientific quality of our manuscript.