Author's response to reviews

Title: Acute murine antigen-induced arthritis is not affected by disruption of osteoblastic glucocorticoid signalling

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Author’s response to reviews: see over
Dear Dr. Cornacchia,

We would like to re-submit/transfer the revised version of our Research Article, entitled

“Acute murine antigen-induced arthritis is not affected by disruption of osteoblastic glucocorticoid signalling”

by Cornelia M. Spies, Edgar Wiebe, Jinwen W. Tu, Aiqing Li, Timo Gaber, Dörte Huscher, Markus J. Seibel, Hong Zhou and Frank Buttgereit.

to *BMC Musculoskeletal Disorders*. We previously had submitted the manuscript to *Arthritis Research & Therapy* (ID 176484870998302; submission date 19 May 2013) and we are pleased to accept the suggestion of the editors to transfer it to *BMC Musculoskeletal Disorders*. In their decision the editors advised us that *BMC Musculoskeletal Disorders* would be happy to consider our manuscript for publication if the peer reviewer comments are addressed and the revisions are made.

We have revised our manuscript according to the comments made by the peer reviewers. These revisions are now incorporated into the revised manuscript. Changes in the manuscript are underlined. Our responses to the specific points are listed below.

**Reviewer I**
The reviewer gave recommendations that were very clear, helpful and constructive to improve the paper. We have addressed the critical points, as suggested in the manuscript. Our reply to the particular comments is as follows:

1.) We chose to use the prolonged AIA model in addition to the traditional AIA model, since acute AIA tends to resolve from day 2 p.i. onwards, and we additionally wanted to study longer-term effects of AIA on arthritis and bone. We induced flare-ups by intravenous re-injections of mBSA, because we intended to keep mechanical damage, potentially induced by repeated intra-articular injections, to a minimum. The method of flare induction by intravenous antigen re-injection has been described by the well-respected group of Wim van den Berg (Refs. 26, 28). The relatively high frequency of
anaphylactic reactions was unexpected. We have now added this information on pages 5-6.

2.) It is now clearly described on pp. 4-5 that the first injection of immunisation was given on day -21, the second injection of immunisation was given on day -14, and day 0 refers to the day of the intra-articular injection.

3.) We show the histomorphometry and micro-CT data for the first experiment graphically, but for the second experiment in the text, in order to avoid overload of the figure, but to provide the data completely.

4.) We found no differences in multiplex analysis of serum cytokine levels between arthritic and control mice for WT mice, and only IL-1\(\alpha\), IL-12 p40 and IL-13 for tg mice. This is in accordance with a recently published follow up (from 7 hours to 14 days) multiplex analysis of 24 cytokines in synovial fluid and sera of rats developing antigen-induced arthritis (Ref. 46). Cytokine concentrations in sera also showed only little variation here, without e.g. any change of IL-1\(\alpha\), IL-1\(\beta\), IL-6 and TNF-\(\alpha\) levels, whereas between cytokine concentrations in arthritic synovial fluid and histological or clinical parameters some correlations were established. We agree with the authors, that “such results are consistent with the local and monarticular nature of AIA, rendering the amount of cytokines produced within a single diseased joint prone to be reduced by degradation in the lymphatic system or by dilution into the bloodstream or both”. We amended this in the discussion section (p. 15).

The relevance of the differences for IL-1\(\alpha\), IL-12 p40 and IL-13 between AIA WT and tg mice remains unclear. IL-12 p40 and IL-13 levels were lower in tg mice in comparison to WT mice after 14 days, but paradoxically higher after 28 days. In our study of K/BxN serum-induced arthritis, IL-12 p40 levels had not been different, and IL-6 and M-CSF levels tended to be altered in tg mice in comparison to WT mice (IL-1\(\alpha\) and IL-13 levels were not determined) (Ref. 8). There is indeed evidence for a (endogenous) glucocorticoid modulation of IL-1\(\alpha\), IL-12 p40 and IL-13 (Refs. 47-50); however, such a modulation in osteoblasts apparently is of no relevance for AIA. We added these considerations to the discussion section (p. 15).

5.) With respect to the numbers we used numbers of n = 14-17 animals per group in the acute AIA model. We used a repeated measures analysis as the sufficient statistical testing for time course analysis. The impression that the WT- and tg-mice in the short-time and long-time experiments react differently to arthritis induction (Figure 1) is due to the lower number of animals in the long-time experiments. We did not increase the numbers for the long-term model or repeat experiments for ethical and economic reasons. As usual for experimental data, we did no power calculation, but assumed that we were able to detect a relevant significant difference with p < 0.05 with numbers n \(\geq\) 4-6 (see also Reviewer 2, point 7.).

6.) As for the acute arthritis studies also for the prolonged arthritis studies littermates were used for WT and tg mice. We amended this in the methods section on p. 6.

7.) The difference in the baseline body weight between WT and tg mice observed in the prolonged arthritis studies has been observed also for younger mice in previous publications (3-, 5- and 7-weeks old mice, respectively) (Refs. 8, 25). This effect is probably due to growth retardation in the transgenic mice and appears to be equalized with increasing age, as we did not detect a difference in the larger group of 11-week old mice in the acute AIA studies.

8.) The assumption of the reviewer is correct: We meant significant knee joint swelling in comparison to controls, based on the repeated measures analysis. We describe these results more clearly on p. 9 and p.11, respectively.

9.) We decided to show all data for future comparisons and as not much is known about it in literature, even if much of the data shows no significant differences.
10.) As recommended by the reviewer, we added more discussion on what some of our results mean to the discussion section, e.g. discussion of the cytokine results and μCT/histomorphometry results (see point 4.) above, and Reviewer 2, point 6.).

Reviewer II
We thank the reviewer for the remarks, that the manuscript is generally well-written and that the conclusion that "the murine AIA is not affected by disruption of GC signalling in osteoblasts" seems solid and supported by the wide range of data pointing in the same direction.

The reviewer gave recommendations that were very precise, mindful, constructive and clearly helped to improve the paper. Our reply to the particular comments is as follows:

1.) We were happy to read that the methods are generally sufficiently detailed and well-described. As suggested we added a brief description of the randomisation procedure to the methods section on p. 5.

2.) The most important variables for the decision to reject the hypothesis were clinical signs and histology. We clarify this at the beginning of the discussion section (p. 13).

3.) We added in the methods section that bone histomorphometry was conducted in single measurements and the region of interest was a 1.5 x 1-mm area of cancellous bone (p. 7).

4.) We amended the description of the micro CT measurement by stating that the slide thickness was 7 µm, the greyscale index was set from 75 to 255 per cent, and 3D-methods were used in the calculation algorithms (p. 7).

5.) Considering cytokine measurements and statistical power, please see above, Reviewer 1, point 4.) and 5.)

6.) We did not measure a decrease of BV/TV or an increase in osteoclast covered bone surface due to AIA-arthritis in WT-mice compared to the control group of WT-mice, probably because μCT and histomorphometric measurements were done at the contralateral tibia. Even if the severity of arthritis at the arthritic knee in this model reached a normal extent, comparable to what is known in literature (Ref. 51), apparently there are no systemic effects on bone due to the monarticular nature of AIA. This is in line with the cytokine measurements (see above). In our studies of K/BxN arthritis we had seen a systemic effect of arthritis on bone in μCT and histomorphometry at the tibia in WT mice, which was prevented in tg mice (Ref. 8). The extent of erosions achieved in the arthritic knee in AIA was low in short and long-term experiments, consistent with previous investigations (Refs. 52, 53). We added these considerations to the discussion section (pp. 15-16).

7.) The impression that the WT- and tg-mice in the short-time and long-time experiments react differently to arthritis induction (Figure 1) is due to the lower number of animals in the long-time experiments. We added this explanation in the discussion section on p. 13 and additionally provide the numbers in the legend of Figure 1 (see also Reviewer 1, point 5).

8.) Day (7-)14(-21) is a usual and common day for the time of analysis in antigen-induced arthritis (Refs. 26, 27). We expect that timing may not have had an effect on the cytokine profile due to the monarticular nature of AIA and according to the literature (see answer to Reviewer 1, point 4.).

9.) We removed the conclusions according to the K/BxN serum-induced arthritis and restricted comparisons to the K/BxN serum-induced arthritis to the discussions section (p. 2, p. 15).

10.) As suggested we emphasized in the title that the AIA model studied is a model of acute arthritis (p. 1).
We rephrased the conclusion in the abstract according to comments 9.) and 10.), respectively (p. 2).

12.) We corrected the typographical errors in Table 1.

13.) We changed the terminology from “unchanged” to “not different” (p. 2).

14.) We removed the denotation of “bone resorption” and “bone formation” in Figure 3.

We would like to thank the referees for the very carefully conducted reviews - our work has clearly been improved due to these reviews. Hopefully, these changes make the manuscript acceptable for publication. We thank you very much for reconsidering our manuscript for publication in *BMC Musculoskeletal Disorders* and look forward to hearing from you soon.

Yours sincerely,

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