Reviewers report

Title: Elevation of Bombina variegata peptide 8 in mice with collagen-induced arthritis

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Reviewer: Rolf Bräuer

Reviewers report:

Bv8 protein as a member of the vascular endothelial growth factor family is involved in different cell functions such as angiogenesis, haematopoietic cell mobilization, hyperalgesia and circadian rhythm regulation. Therefore, it is an interesting candidate as a mediator of inflammatory processes and as a therapeutic target. The authors carried out a study to determine whether this factor may play a role in the pathogenesis of arthritis. For this purpose, the murine collagen-induced arthritis (CIA) was used, a well established experimental model of human rheumatoid arthritis. The severity of arthritis was evaluated by repeated scoring of the swelling and deformities of wrist and ankle joints. Furthermore, RNA was extracted from joints at different time points of arthritis development and examined for Bv8 mRNA expression by quantitative RT-PCR, the protein expression was analyzed immunohistochemically in synovial tissue and bone marrow. It was shown that Bv8 is expressed/increased in synovial tissue and bone marrow of arthritic mice and that there is a strong correlation between the Bv8 expression and the severity of arthritis. In the present study the authors address a very important clinical problem namely the characterization of mediators which are involved in or responsible for the pathogenesis of chronic arthritis. However, in my opinion, the results presented here are somewhat preliminary; the study might be essentially improved by more detailed experiments and more thorough evaluation of the data.

The following suggestions should be addressed:

Major compulsory revisions:

1. It is not clear for me, which experimental model was used; from the manuscript, it seems that the classical CIA model was used (immunization with collagen type II in CFA), however in the cited reference the passive antibody-mediated model is described. Please clarify this essential question and describe the experimental conditions in a more detailed fashion.

2. For a thorough evaluation of arthritis, the authors should include the histological evaluation of inflammation and joint destruction (besides the clinical scoring), particularly because HE stained slides are already available from day 28 of arthritis.

3. The immunohistochemical characterization of cells expressing Bv8 in synovial tissue and bone marrow should be improved, e.g. by double-staining or by
staining in serial slides (if essential antibodies do not work on fixed, decalcified paraffin-embedded material, the immunohistochemistry is also possible on cryostat sections of unfixed, undecalcified whole joints: van Norden et al. Histochemistry 1986;86:127).

Minor essential revisions:

4. In Fig. 1a and 1c, the number of animals (n) for each time point should be included in the legend; in Fig. 1c, the description of the units on the ordinate is missing.

5. In my opinion, the Fig. 3a and 3c are not representation of bone marrow in normal (uninvolved) joints. The authors have to explain how they guaranteed standardized bone marrow sampling from the same anatomical region in order to obtain comparable sections in normal and CIA mice (see Fig. 3).

Discretionary revisions:

6. To receive some more information on whether Bv8 expression is involved in the angiogenesis process, the density of vessel surface within the inflamed tissue should be estimated immunohistochemically (to clarify the functional of Bv8 in arthritis, even better might be in vivo experiments using anti-Bv8 antibodies or siRNA).

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests