Reviewer’s report

Title: Lipopolysaccharide treatment protects B10.BR male mice from spontaneously developing ankylosing enthesopathy: the potential role of interleukin-10

Version: 1 Date: 2 October 2011

Reviewer: Stephanie Shifra Weinreich

Reviewer’s report:

Major compulsory revisions:

1. Methods, sections on mice and serum samples: it is unclear which mice were used for the serum studies, because it is unclear how and when their blood was collected. Was the bleeding done at sacrifice, i.e. around the same time as splenectomy? This confusion could be addressed with a figure or table, showing the flow of which mice underwent which intervention at which time.

2. In Results, paragraph 5 (‘In vitro cytokine response of spleen cells’) the relevant text, including description of Figure 6, is missing. It seems that the text of the previous paragraph was copied.

3. The paper is inconsistent in its handling of the finding that one of the 55 LPS-injected mice developed ANKENT (Results, paragraph 2). The abstract gives the impression that this group did not develop ANKENT at all. Similarly, paragraphs 2 an 4 of the Discussion section include statements that LPS-injected mice did not develop ANKENT. A consistent approach should be followed. Do the authors speculate that the protective effect of LPS had subsided in the mouse which developed ANKENT at age 9 months? This should be discussed explicitly.

4. Currently lacking in the paper is reflection on what it means to compare immunological profiles on a group level, considering the heterogeneity within groups. In other words, how valid is it to consider the immune profile of the PBS-treated group as ‘typical’ for ANKENT susceptibility, if the cumulative incidence of ANKENT only reached 14,2% in this group?

5. The Discussion section, paragraphs 5 through 12, is difficult to follow. The paper seems to struggle to develop an explanation for how LPS might protect against ANKENT.

The paper discusses in turn each of the group-level immunological differences observed after the 2nd and 4th LPS injections (at about age 4 and 5 months) and also at age 10 months. Sometimes small, inconsistent or even absent effects are given a surprising amount of emphasis. For example, it is unclear why the role of Tregs in allergy is even mentioned. Furthermore, the comparisons of cytokine data from serum and LPS-stimulated spleen are patchy. Also, these two sets of observations are not addressed from a theoretical point of view. What would the
authors expect from LPS-stimulated spleen cells from a never, once or twice LPS-challenged mouse at various ages? Were the authors surprised at the discrepancy in IL-10 data between serum and LPS-stimulated spleen cells? Finally, the discussion of TNF-alpha in paragraphs 9 and 11 seems to be contradictory.

6. Due to the unexpected findings (first-ever observation of a protective effect of LPS) and the uncertainties of integrating observations from groups of mice where randomization may have been compromised (see point 9 below), I recommend shortening and restructuring the second half of the discussion section, starting from paragraph 5. The authors might focus first on the immunological effects of LPS treatment as measured on cell subpopulations and cytokine expression, tying together the strongest and most consistent effects and discussing them in relation to the literature. Next, the authors might speculate on how these immunological data might reflect a possible protective effect of LPS on ANKENT susceptibility, keeping in mind the limitations in the current experiments (possible non-comparability of groups, heterogeneity within groups, and the limited number of timepoints when immunological parameters were measured). The current version of the paper mentions many analogies to human disease and animal models; more focus might make the paper stronger. For example the conclusion mentions the hygiene hypothesis but it is not discussed at all.

Minor essential revisions:

7. In several places the paper refers to numbers of cells, instead of percentages (or proportions), e.g. in the abstract under results. Similarly, in the Discussion, paragraph 6, it is inaccurate to speak of ‘expression of … cells’.

8. The mice shown in Figures 1 and 2 should be identified in relation to the experiments in this paper.

9. Since various non-genetic factors have been associated with risk for ANKENT (caging, reference 33; maternal age, see Weinreich et al. 1995; Annals of Rheumatic Diseases 54:754.) it is relevant to describe more details of how mice were divided in groups, i.e. how they were randomized and housed. Were litters split? Were all mice always caged with other males? Was the experiment for observing ANKENT done simultaneously with the experiment which measured immunological parameters after the second and fourth LPS treatment? Such details will help the reader to form an impression of the validity of comparisons between LPS and control groups as well as the validity of integrating data from mice observed for ANKENT with data from mice observed only for immune parameters.

Discretionary revisions:

10 Can the authors clarify why the numbers 55 and 70 were chosen?

11. The Methods section, subsection Statistical analysis, first paragraph states that p values ‘within the limits p<0.01-p<0.05 were considered significant’. What is the reason for giving a lower limit? The second paragraph of this section states
that standard errors are shown for cell populations. It is more appropriate to show standard deviations. The authors might actually consider showing all the data points, see for example figure 9.7 in D.G. Altman, Practical Statistics for Medical Research, Boca Raton, Chapman and Hall, first edition (1991).

12. It seems that the cytokine production by spleen cells fluctuated a lot, i.e. Figure 6 shows that the average IL-6 values for control mice were about 70, 40 and 200 pg/ml at different time points. Do the authors wish to speculate about the significance of this fluctuation?

13. This paper is the first report that LPS may protect mice from developing ANKENT, at least for a certain period of time. This was quite unexpected and therefore the authors are not to be blamed for the fact that the immunological observations were not optimally timed to explain such a phenomenon. However, it would be interesting if the authors could briefly propose a direction for future work. For example, do the authors see merit in repeated measurements of serum IL-10 in cohorts of LPS-treated and control mice, during long-term observation for ANKENT, as IL-6 was measured in reference 11? Do they see value in functional immune assays?

Minor comment:
14. Reference 33 does not present any data about aggression, as the authors imply in the Discussion section, paragraph 3.

15. In a few places the English is a little awkward, e.g. in the first sentence of the abstract (‘..ANKENT represents one of animal models...').

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests.