Author's response to reviews

Title: Tracking the transcriptional host response from the acute to the regenerative phase of experimental pneumococcal meningitis

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Author's response to reviews: see over
Re: MS: 8804221683029975 - Tracking the transcriptional host response from the acute to the regenerative phase of experimental pneumococcal meningitis

Thank you for the opportunity to submit a revised version of the above mentioned manuscript. We would like to extend our thanks to the reviewers for their thoughtful comments that helped us to improve the manuscript. Following the suggestion of the reviewers we complemented the current version of the manuscript by the inclusion of additional experimental data, figures, text and references. Thus we hope to have addressed all concerns raised by the reviewers as stated in the rebuttal text submitted separately. The changes to the manuscript are listed in the point-by-point reply to the critiques by the reviewers:

Reviewer 1

Major compulsory revisions:

1. Results section, page 5. The “Clinical parameters of meningitis” paragraph appears limited in its current state and results are not illustrated in any table or figure. It is obvious that the authors have extensively published on this subject before, but there are no references to past work to justify the paucity of detail included here. Please extend this section and cite any relevant past papers, if results are exactly the same as those observed in previous manuscripts. For instance, be more exhaustive with respect to “altered state” and “reduced weight gain”. What are the actual values and percentages? A table would probably be ideal to summarize the clinical parameters. This table could also contain the median scores of apoptotic cells reported in the next paragraph.

2. Results section, pages 5 and 6 “Inflammatory parameters”. Similarly to the previous comment, it would be extremely useful and important to see the cytokine data summarized in a figure or at least in a table. The cytokine profile was conducted by Luminex analysis, extensively described in the Materials and Methods section, but results are not reported with appropriate detail.

Answer to 1 & 2: We agree with the reviewer’s comments and have implemented the suggestions by addition of 2 tables.

Measures: A table summarizing the clinical parameters at the three examined time points after infection was included (Table 1). Likewise a table summarizing the Luminex data was added (Table 2).

3. Results section, page 6. Are results in the PCR paragraph reported or summarized anywhere? There is no reference to the primers in Table 1 here, and in fact Table 1 is never mentioned in the text of the manuscript. Please resolve.
Answer: We thank the reviewer for pointing out this omission.

Measure: Table 3 (former table 1) was expanded with the respective PCR and Chip expression ratios. The table is now referenced at the end of the PCR paragraph in the Methods section and in the Results part.

4. Results section, pages 10 and 11. It is unclear why figures were made for gene expression in the hippocampus, but not for the cortex. Please resolve or explain.
Answer: In comparison to the Gene Ontology annotation in the hippocampus, there were few and rather unspecific biological processes overrepresented in the cortical tissue. Furthermore the analysis of the constructed GO graph revealed that the identified processes are solely connected through very generic parent terms. These findings imply that besides the processes of inflammation and immune response affecting both brain structures upon infection there are no distinct disease mechanisms in progress in the cortical tissue. For these reasons we focused on the processes identified in the hippocampus for illustration in figures 4 and 5.

5. Results section, pages 11 and 12. In the first part of the “Time and tissue intersection” paragraph, I am assuming that the text is referring to Figures 6 and 7. Please cite appropriately or explain.
Answer: The first part of the “Time and tissue intersection” paragraph describing the expression changes of transcripts involved in the activation of the innate immune response are not represented in a figure. These mechanisms are well described in other excellent publications and reviews in the field that are references in the paper. Due to the complexity of the gene regulatory network that is activated by the disease our intention was to emphasize to our knowledge new transcriptomic aspects of pneumococcal meningitis and keep the paper “readable” and “digestible”. Therefore the focus of this paragraph lies more on the activation of genes regulating the microglial response in the light of neuronal damage and –protection which is illustrated in figure 9. Figures 6 and 7 showing the regulation of genes involved in the neurogenic TGFbeta and wnt signalling pathways are referenced in the section “Genexpression in the hippocampus at 72 h after infection”.

Measures: Additional references covering the aspect of innate immunity in bacterial meningitis were added to the “Time and tissue intersection” paragraph of the Result section.

6. Results section, page 12. Please report the findings illustrated in Figure 9 in greater detail, indicating what factors are more important in each tissue at 1 and 3 days after infection.
Answer: It has been shown, that even focal infections of the brain lead to an extended microglial activation which is not limited to specific brain structures. This notion is also reflected by our data showing that genes involved in microglial regulation have very similar expression kinetics in the cortex and the hippocampus at 24h and 72h after infection. Due to this similarity a weighting of the importance of single factors of microglial regulation in the two examined brain structures is not possible on the sole basis of transcriptomic data.

Measures: Additional papers describing the extensive microglia activation upon bacterial meningitis were added as references in the Discussion section page 16.

7. Discussion section, page 13. In the part regarding the use of 3 animals per time point, there is appropriate discussion of the control of sample variability. Would the use of more animals eliminate the need for this type of control? Please comment in the discussion.
Answer: We agree with the reviewer that this point merits additional explanation.

Measures: The following paragraph has been added to the discussion section on page 13-14: “Experimental in vivo models of infection inherently show a variability in clinical severity of disease...
and the corresponding disease parameters e.g. pathogen burden, inflammatory cyto-/ and chemokines, and tissue destruction. Inter-individual variability in disease and the subsequent readouts i.e. apoptosis occurs also in the present experimental model of BM. Therefore we regard this kind of standardisation as mandatory also with larger sample groups to avoid an increase in gene expression variability, which may hamper the detection of minor but still biological significant expressional changes."

Minor essential revisions
1. Abstract, Page 2. Please add the “Methods” subtitle to the abstract.
Done

2. Abstract, Page 2. In the “Results” subsection please specify the tissue types (cortex and hippocampus) studied, instead of vaguely referring to “both tissue types…”
Done

3. Results, page 7. Cite figures and panels correctly and consistently. For instance, in the “Spatial and temporal gene expression” section, add “Figure 2A” after 2974 transcripts. “Figure 1A” is the incorrect citation here, please change.
Done

4. Results, pages 8 and 9. Remove “Figure 4” and “Figure 5” next to the paragraph titles.
Done

5. Discussion, page 15, line 3. Please fix the incomplete sentence, “furthermore there is evidence…”
Done

6. Discussion, pages 18-21. The “Regulation of the host defence: tissue and time intersection” paragraph is repeated twice. Please remove.
Done

Done

8. Materials and methods, page 32. Please specify volumes for tissue homogenization. Answer: Thank you for pointing out this “orphaned” text fragment. Measure: Since we didn’t show any western blot data we removed the “Tissue extracts for BCA assay and Westernblot” paragraph.

9. Figure legend 1, page 39. Please add more information to this legend. This should be a guide for a better understanding of a fairly complex experimental overview.
Done

10. Figure legends 5 and 6, page 40. Please expand both legends.
Done

11. Please check throughout the manuscript the use of italics for Streptococcus
pneumoniae.
Done

12. Please check throughout the manuscript the use of commas and punctuation in general. This will greatly improve the text flow.
Done

Reviewer 2
No actions requested.

Editorial Requests
The revised manuscript contains a method section and a statement of competing interests.

The Microarray data is deposited and curated at the ArrayExpress platform [http://www.ebi.ac.uk/microarray-ae/](http://www.ebi.ac.uk/microarray-ae/). The accession number is supplied on receipt.

We hope to have addressed the referee's points satisfactorily with the outlined changes, so that the manuscript is now acceptable for publication. The current re-submission of the revised manuscript is in full compliance with the published editorial policy of BMC Infectious Diseases.

All inquires regarding the manuscript should be addressed to the undersigned at the address in the letterhead.
Sincerely yours,

Stephen L. Leib