Author’s response to reviews

Title: Transcriptome analysis of human monocytic cells infected with Burkholderia species and exploration of pentraxin-3 as part of the innate immune response against the organisms

Authors:

Sophie Aschenbroich (aschenbroich@wisc.edu)

Eric Lafontaine (elafon10@uga.edu)

Maria Lopez (mclopez@ufl.edu)

Henry Baker (hvbaker@ufl.edu)

Robert Jeffrey Hogan (jhogan@uga.edu)

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Author’s response to reviews:

Dear Reviewers,

We thank you for reviewing our manuscript, MGNM-D-19-00065, whose title we have revised to “Transcriptome analysis of human monocytic cells infected with Burkholderia species and exploration of pentraxin-3 as part of the innate immune response against the organisms.” Please see our answers to your comments below. Please, also find two different copies of the manuscript for ease of review, with one copy displaying tracked changes made to address reviewer comments and the other copy with all modifications having been made without tracking of changes ("final version").

Technical Comments:

1. In line with our submission guidelines [https://bmcmedgenomics.biomedcentral.com/submission-guidelines/preparing-your-manuscript/research-article] please include a 'Methods' paragraph in the Abstract, to briefly describe how the study was performed and the statistical tests used. Please take into account that the length of the full Abstract should not exceed 350 words.

->Thank you for bringing this to our attention, a Methods section and statistical analyses used had been added to the abstract.
2. Please move the 'Methods' section before the 'Results' in the main manuscript text.
   ->This has been modified.

3. Please provide figure titles/legends under a separate heading of 'Figure Legends' after the References. If Figure titles/legends are within the main text of the manuscript, please move them.
   ->This section has been added.

Figure files should contain only the image/graphic, as well as any associated keys/annotations. If titles/legends are present within the figure files, please remove them.
   ->This has been addressed.

Figures should be provided as separate files, and each figure of a manuscript should be submitted as a single file. Figures and Tables have been removed from the main manuscript and are included as separate files.
   ->The two tables and 10 figures have been removed from the manuscript and saved independently.

Please ensure that all figures/tables and supplementary files are cited within the text. Any items which are not cited may be deleted by our production department upon publication.
   ->This has been addressed.

4. Please provide a list of all the abbreviations used in the manuscript. This list should be placed just before the Declarations section. All abbreviations should still be defined in the text at first use.
   ->Abbreviations have been added.

5. In the 'Funding' statement, please declare the role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.
   ->This information has been specifically added.
Reviewer reports:

Narisara Chantratita (Reviewer 1):

The objective of this study is to investigate the potential for modulation of host immune response-related genes and pathways during intracellular survival of B. mallei by assessing global host transcriptional changes during infection of a human Mono Mac-6 monocytic cell line. This research demonstrates the interactions between PTX3 and Burkholderia species including B. mallei, B. pseudomallei and B. thailandensis.

Comments

1. Abstract: Please include the results of rhPTX-3 binding to bacteria and complement activation.

->We thank the reviewer for this comment and have briefly included these results and made additional adjustments to the abstract in order to adhere to the strict 350 word limit.

2. The sentence in the abstract "We report on PTX3 as a novel opsonin of Bt and Bp, together with evasion of PTX3 recognition by Bp" is overstated. In the results PTX-3 has just been demonstrated to bind to some of these bacteria. The term "opsonin" may be misleading because this study does not show that PTX-3 facilitates phagocytosis. The conclusion should be modified.

->We thank this reviewer for their comment; the wording has been modified in multiple locations (the abstract, results, figures 9-10 have also been modified, discussion, and conclusion sections) to clarify the nature of the binding interaction.

3. In several places, PTX-3 has been demonstrated to be involved in innate immune recognition, phagocytosis and complement activation in several microorganisms. Please provide example names of those organisms in the introduction.

->Specific examples of organisms have been added to the background section.

4. The experiment in Figure 5 is "binding assay" not "opsonization". Please consider to change.
As recommended by the reviewer, we have modified the title of this figure to “Human PTX3 recognition of select Burkholderia sp.”

5. In discussion, the authors could make it more concise and avoid to over interpreting the data. There are many sentences throughout this part that the suggestions are not supported by the data in this study.

We appreciate the reviewer’s comments on the discussion section and have made multiple modifications to address these concerns including clarifying the nature of the binding interaction and better highlighting that speculations on the data would need further exploration to substantiate.

David DeShazer, Ph.D. (Reviewer 2): This manuscript describes Burkholderia mallei (Bm) and Burkholderia thailandensis (Bt) uptake, replication and transcriptional modulation in the human monocyte Mono Mac 6 (MM6) cell line. Both species replicated inside MM6 monocytes and resulted in the up-regulation of the Pattern Recognition Receptors (PRR) pathway, including pentraxin-3 (PTX3). Interestingly, PTX3 opsonized Bt and Burkholderia pseudomallei (Bp), but not Bm.

Unfortunately, the authors were unable to identify a definitive functional activity for PTX3 using a variety of well-controlled in vitro assays with Bt. The data presented regarding the interaction of Burkholderia sp. with human monocytes and PTX3 is novel and of interest to the melioidosis and glanders research communities, but the abundance of negative data is somewhat disappointing from a reader and reviewer standpoint. Additional experiments that might benefit this manuscript include the following: 1) explore the PTX3-Burkholderia interaction in the presence of human neutrophils, 2) further characterize immune response pathways that are differentially regulated following Bt an Bm infection of monocytes, 3) identify how PTX3 binds specifically to the surface of Bt and Bp, but not Bm or 4) examine the relative susceptibility of PTX3 knockout mice following infection with Bm and Bp.

Rebuttal: We thank the reviewer for their suggestions and comments. The primary focus of this manuscript is to report on which host genes are modulated during infection with the overarching goal of comparing and contrasting results not only between Bt, Bp, and Bm, but also at multiple time points. We sincerely appreciate the reviewer’s suggested follow-up experiments, and 3 of the 4 suggested approaches are already planned for exploration. Given the size and scope of the
current work, we are planning to publish these results in one or more additional, stand-alone manuscripts.

Specific comments

1) Line 24. It is not clear that PTX3 is a "critical" component of the host innate immune response against Bm, Bp and Bt. Perhaps modifying the title would be appropriate until there is a definitive function for PTX3 in controlling infection with these organisms.

-> We appreciate the reviewer’s suggestion and have modified the title to better reflect the data we have thus far.

2) Line 47. The first table that is presented in the text is Table 2. Perhaps Table 2 should actually be Table 1?

-> We thank you for catching this typographical error and have modified accordingly.

3) Figure 3. Given the different host virulence phenotypes of Bm and Bp, it seems that the authors should have commented on the potential pathogenic importance of the differentially regulated pathways in monocytes infected with these species. Otherwise, this figure could probably be eliminated from the manuscript. For example, the Th1 pathway appears to be differentially down-regulated early in Bm-infected monocytes. It would also be easier to interpret this figure if Bm and Bt pathways could be aligned.

-> The authors agree wholeheartedly that aligning the pathways would be most beneficial to the readership. However, due to limitations inherent to the IPA software, the authors are unable to modify the figure in this manner. In an attempt to make the figure more digestible to the readership, we have indicated in bold pathways that are different for Bt or Bm infections across 2 time points so that the readership can more easily discern major pathway differences. We would prefer to keep this figure within the manuscript, as it nicely highlights the directionality of the Pattern Recognition Receptor Pathway, among other host canonical pathways that may be worthy of further exploration for other researchers in the field.

4) References. Please italicize the genus and species names in all references.

-> These changes have been made.
5) All references that I find for the Mono Mac 6 cell line italicize it as "MM6" rather than "Mm6". Perhaps this could be modified to be more consistent with the literature?

->These changes have been made.