Peer Review File

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Reviewer A

Comment: Some possible points to be discussed too. A good, balanced review though.

Regarding mechanisms in CD; Bannatine et al has suggested increased intestinal permeability? Also relevance of Granuloma formation

Reply: We thank the reviewer for their thoughtful comments. We read through the Bannatine et. Al paper and included a point regarding increased intestinal permeability in lines 99-101.

Comment: Can MAP be described as an opportunistic infection and TB more pathogenic? Hence the different responses to immunosuppressive therapy.

Reply: We would not think of MAP as an opportunistic infection in terms of thriving in the setting of immune suppression. Many CD patients have MAP present, even before they receive immune suppression. It is possible that it is playing a bystander role rather than pathogenic, as we have outlined elsewhere in the manuscript.

Comment: Need for greater combination (and difficulty) for treatment of atypical mycobacterium with high levels of resistance than that seen in TB.

Reply: We thank the reviewer for this point. We have added a sentence in lines 171-174 stating the difficulty of eradicating MAP and requirement of multidrug regiments with high resistance, and the fact that none of the drug studies confirmed MAP eradication. We would also point to the MAP US trial, highlighted in lines 188-191, which formulated RHB-104 combination pill using culture/sensitivity data of MAP.

Comment: Concept of colonisation vs infectivity vs disease expression.
e.g. high rates of TB infection but only a small proportion manifest disease

Reply: We agree with the reviewer that is unclear if MAP is playing a bystander role or is actually pathogenic. We believe this has already been addressed in the manuscript including lines 124-132 and 228-231.

Comment: Need for culture-based assay over molecular diagnostics given differing activity of the microorganism. See the difficulty of screening for TB – normally triple testing – CXR, IFGN, Sputum testing/ Mantoux.
Molecular tests only detect presence of organism. Not activity.

Reply: We agree with the reviewer that merely the presence of MAP does not indicate it is playing an active role in the disease process. Need for improved diagnostics is very important. We did highlight several studies that used a MAP specific immune response showing there was likely some activity of the organism. We highlighted this in lines 128-132.

Comment: Right combination of antibiotics – limitations on sensitivities knowledge.

Critical need for diagnostics of MAP in treatment trials even with antibiotics, vaccines etc.
Reply: We agree with the reviewer and have highlighted this limitation in the manuscript. Please see lines 168-174 as an example.

Comment: Are RCTs the way forward or Prospective Observational Trials first? Signal differences, cheaper etc.
Reply: We feel that more randomized controlled trials that demonstrate the baseline presence and subsequent eradication of MAP following treatment are needed. We added this statement in lines 219 to 220.

Reviewer B

Comment: This is a very well written mini review describing the potential role of Mycobacteria avium subsp. paratuberculosis (MAP) in Crohn's Disease (CD). The authors provide an overview of the historic and current thinking on this topic, followed by a more detailed discussion of the role of anti-MAP therapy in the management of CD, and how this may implicate MAP in CD aetiopathogenesis.
This is a useful review that would be of benefit to those interested in the evidence of anti-MAP therapy in the management CD. Beyond this, the other topics covered by this review, although nicely summarized, have been discussed more extensively elsewhere in recent publications.
Some minor revisions are required prior to publication. One of the purposes of narrative reviews, such as this, is to provide readers with references for further information. Outside the focus of this review (anti-MAP therapy for CD) secondary referencing in commonplace and inappropriate sources are cited to support many of the key points (see specific examples below). Furthermore, journal guidelines limit the number of references in unstructured, mini-reviews such as this to 30. With these two points in mind, I would advise reducing the scope of this review to focus more on anti-map therapy, rather than summarizing the whole topic e.g. the sections regarding historical origins, the transmission of MAP, etc. could be removed. This would allow for a more detailed discussion regarding the strengths, weaknesses, and implications of the evidence around anti-MAP for CD, which would be the most useful contribution to the existing scientific literature.
Reply: We thank the reviewer for their thoughtful comments. We do agree that the focus of our manuscript was to summarize the current data regarding anti-MAP therapies in CD patients. We have included all case series, prospective and retrospective cohort studies, and randomized controlled trials that we are aware of and summarized the results into Table 1. We feel, however, that the reader needs to understand the historical context and the basic rationale as to why eradicating MAP can theoretically alter the disease course in Crohn's disease, as this treatment remains very controversial among clinicians. We believe this is essential prior to discussing the available clinical/ treatment data.

Comment: L47-48: Please find a more appropriate citation to support the statement: "Since the late nineteenth century, Mycobacterium avium subspecies paratuberculosis (MAP), is known to be involved in the pathogenesis of JD" - the current citation is an editorial discussing how to establish the role MAP may have in CD and does not support this statement.

Reply: Thank you for pointing this out. We edited the reference to be from Professor Dunkin's 1936 article about the topic.

Comment: L57: I read this sentence to refer to mycobacteria (i.e., plural) rather than a singular mycobacterium.
Reply: Thank you. We edited this to say "mycobacteria."

Comment: L67: Need a period at the end of this sentence.
Reply: Thank you. We added the period.

Comment: L84. Describing the organism as "present" in milk, water, and infant formula needs qualification as all cited studies only found MAP in a proportion of tested samples. This is still relevant but it is important not to give the impression it is universally present in these samples. "The organism is… present in pasteurized dairy milk, …" is misleading and not supported by the provided citations. For example, one of the citations (Grant et al) found viable MAP in 1.8% of pasteurized milk samples. You should be clear about whether studies have detected MAP DNA with PCR or viable MAP with culture. Of course, you may consider non-viable MAP to be important in the pathogenesis of CD, in which case this is worth discussing explicitly. It is also important to acknowledge the imperfect specificity of IS900 PCR due to the homology of this region with other Mycobacterial spp.
"The organism is… present in … water supplies" is not supported by the provided citation which is an editorial discussing how to establish the role MAP may have in CD.
Reply: Thank you for the comments. We have updated this section to provide more specific information that is consistent with the cited sources. We now only included numbers reflected viable MAP cultures and gave percentages instead of saying that MAP was "present."

Comment: L86. It is not correct to conflate the survival of MAP with replication, MAP can survive in the environment for a long time.
Reply: We changed the word "survive" to "replicate."

Comment: L88. This citation (Honap et al) is a review of anti-MAP therapy for CD and is not an appropriate reference to support the preceding statement.
Reply: We believe that Honap et. al is an appropriate reference for the first half of this sentence, but agree that it does not cover the part about pasteurization. As such, we have edited the pasteurization portion of the sentence to be a reference from the Grant et. al paper.

Comment: L88. "As such, MAP is likely highly prevalent in the human population." This is conjecture and needs to be supported with appropriate references. The following sentence refers to a single study that reported a prevalence of 8.4% - can this be considered "highly prevalent"? The logic of "high dairy herd prevalence" plus "resistant to pasteurization" equals "high prevalence in human population" does not hold because a MAP-positive herd frequently has very few animals that are shedding MAP.
Reply: Thank you for this comment. We edited this sentence to demonstrate that although this is a

single study, it is notable for its very large sample size. We further clarified that this did not indicate that this data does not show active intestinal infection, it demonstrates a substantial portion of people in India has had likely MAP exposure.

Comment: L108-100. This feels like cherry-picking the study with the most extreme effect size to discuss, at the very least some reference to the sample size would be advisable. Similarly, it is not appropriate to (seemingly) base one of your concluding remarks "MAP is detectable in the majority of patients with CD" (L202) on this single study when the other studies you reference indicate more CD patients did not have detectable MAP than those that did. It would be more appropriate to reference OR (or similar) in this case.
Reply: Thank you for this comment. We edited it to highlight that other studies have had found a difference in prevalence, though to a lesser degree.

Comment: L212. The word "the" is repeated.
Reply: We deleted the additional "the."

Comment: L217. Clinical Johne's Disease in cattle is limited by surveillance and pre-emptive culling of infected cattle so this figure is difficult to ascertain. Regardless, the supporting reference is inappropriate.
Reply: This one is all you Dana.

Comment: Table 1. This is an extremely useful summary table for readers, but it needs to be referenced and referred to in the text.

Reply: Thank you. We added a sentence to reference the table in the text.

Reviewer C

Comment: Specifically, with regards to some of the second reviewer's suggestion:
I agree that the manuscript could provide more detail specifically on the topic of MAP treatment and CD outcomes.
Reply: Thank you for this comment. We included all of the clinical studies regarding MAP treatment in CD outcomes that we could find in our literature review. This includes case series, prospective/retrospective cohorts, and randomized controlled trial. We acknowledge that this data is relatively scarce.

Comment: I would however NOT exclude, but rather shorten/edit the background historical information on MAP.
I believe that for a general audience it provides necessary context for the treatment information that follows
Reply: We agree with this reviewer that the historical context is important to this debate.