Reviewer’s report

Title: Presence of intestinal Mycobacterium avium subspecies paratuberculosis (MAP) DNA is not associated with altered MMP expression in ulcerative colitis

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Reviewer: Brian Bressler

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The manuscript submitted by Rath et al., deals with measurement of mycobacterial DNA in biopsies of IBD patients. In addition, authors correlated these findings with the transcription profile of metalloproteases involved in recycling of matrix in intestinal cells.

This paper provides additional data about MAP as the etiological agent of CD. Although the authors conclude that an upregulation of the MMP family was not observed, they should be very cautious about these conclusions in the manuscript for the following reasons:

Main concerns:
- It looks that authors did not choose the right MMP to analyze. Reference number 16 is a key citation in the submitted manuscript. This reference clearly states that mycobacterial infections induce specifically MMP#9. Authors are required to provide data about this specific protein, as they only analyzed #1, 7, 13, 19 and 28. MMP#9 is regulated by TNF-a, IL-18 and IFN-g. Showing the profile of these cytokines also will support the data about up or down regulation of MMP9.
- The transcriptional analyses should be in a histogram, expressing the fold induction in the y-axis as the ratio between infected areas/non-infected areas.
- It is known that spheroplasts are the form of MAP once isolated from human tissues. It should be very well stated in the manuscript that: (Page 10) Chiodini et al, found presence of MAP after 2 years culture, therefore, please change “none of MGIT samples was positive…” to “none of the MGIT samples was positive for the period of 52 weeks…”.
- In materials and methods, it is not described whether patients were under antibiotic treatment. Data corresponding to these patients (if any) should be excluded. Authors should state what kind of anti-inflammatory therapy the patients received (according to Table 3 at the bottom), as drugs affecting the profile of the above mentioned cytokines, it will change/affect drastically the level of expression of MMPs. Therefore, showing an independent analysis of the “none” group n=14 is important for the analysis of the data, and then compare this group with the other 2 receiving AIT.
- Page 12 the statement “which ensure a highly sensitive and specificity approach” should be changed. Although the approach is sensitive, the portion
used for the biopsy does not assure that DNA of MAP is still there. Therefore, the sentence should be “weaker”.

- In the discussion section, authors did not mention about the possibility that MAP down regulates the expression of MMPs. This down regulation can be beneficial to MAP in the first stages of invasion in order to establish a successful infection. Inflamed areas might not been beneficial to the bacterium, as the presence of high concentrations of cytokines will induce tissue destruction which eventually will affect the survival of the pathogens, especially that it is unprotected as a result of cell wall shedding.

Minor comments:

1. Please correct the following:
   - Page 3. Cows are ruminants, therefore the sentence should be changed to something like: in ruminants like dairy cows and many other species…
   - Zn 2+ should be corrected to Zn2+.
   - A reference should be added after extracellular matrix (ECM).

2. Page 5. The paragraph starting with “Each biopsy sample from …” should be clarified whether it includes IBD and non-IBD patients, or it is a mistake in the number. In the same paragraph, “cultural investigation” should be changed.

3. Page 7. ANV should be clarified.

4. Page 8. Please define UNG.

5. Page 9. Please add *n* to the end of Man (n)-Whitney test.

6. Figures 2, 3, and 4 can be presented as a panel of figures.

7. Figure 2. X-axis title, please add *e* to MAP positiv (e).

8. Figure 1 is not necessary for the manuscript. Please remove.

9. Figure 4 is mislabeled as Figure 3.

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

Quality of written English: Acceptable

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' below. If your reply is yes to any, please give details below.