Reviewer's report

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

Version: 1  Date: 29 November 2010

Reviewer: Robert Greenstein

Reviewer's report:

Reviewer. Robert Greenstein
Journal: BMC Gastroenterology
Title: Presence of intestinal Mycobacterium avium subspecies paratuberculosis (MAP) DNA is not associated with altered MMP expression in ulcerative colitis
By: Timo Rath et al.

Thank you for requesting my review of this well performed study of the presence of several antibodies in a variety of gastro-intestinal diseases.

The authors had a dual intent in this study:

Objective #1 was to;
determined the prevalence of MAP DNA in biopsy samples of patients with UC and CD as well as in patients without IBD using highly sensitive and MAP specific PCRs.

Objective #2 was;
To analyze a potential regulation of MMP expression by MAP in vivo, we further assessed the colonic expression of a broad MMP spectrum in UC patients with and without intestinal MAP DNA detection (Matrix Metalloproteinases (MMPs) are a family of Zn 2+-dependent endopeptidases that are considered to be the most potent proteases in the turnover of the extracellular matrix)

For Objective #1, the authors:
determined the prevalence of MAP DNA in biopsy samples of patients with UC and CD as well as in patients without IBD using highly sensitive and MAP specific PCRs.

For Objective #1, the authors additionally attempted to culture MAP from some samples
Samples for cultural investigation were not frozen at any time.

For Objective #2, the authors:
To analyze a potential regulation of MMP expression by MAP in vivo, we further assessed the colonic expression of a broad MMP spectrum in UC patients with and without intestinal MAP DNA detection.

The data for Objective #1 show a non-statistically significant:
more frequent detection of MAP DNA in UC patients (20%) and controls (33%) compared to patients with CD (7%)

The data for the secondary Objective #1: Culture of MAP from samples are not presented, or if they are I could not find them!!!! In attempting do so I searched the pdf file sent to me using the word “culture.” I found the word “Culture” a total of 12 times. Six times in Methods for “Culture”, Twice in the Methods “DNA extraction” section, twice in the Discussion section and twice in the References. There are NO results that I could find!

The data for Objective #2 are much more difficult, if not impossible, to interpret. This is because of the finding presented from Objective #1:

As MAP DNA was detected in only one CD patient, we were unable to analyze MMP expression with regard to MAP status in the CD patient cohort.

Reviewer’s overview of the data presented and it significance:

The authors have spent considerable time and effort on this protocol and the manuscript. They have been diligent and performed a complex set of studies and experiments. They are obviously applying the Null hypotheses and are to be commended.

As presented this manuscript perpetuates a series of publications on the potential zoonosis of MAP that lack clarity and simply muddy the issue.

I have spent a considerable time on this review, as I believe that the effort already expended by these authors merit such a commitment.

I will suggest what I think are the potential enormous payback that might be achieved. However, these suggestions will require more analyses and experiments.

Reviewer’s comment on the global design and concept of this study:

It should be made clear at the outset of these comments, that this reviewer is seriously concerned that MAP is zoonotic and that CD may merely represent the tip of the iceberg of the diseases that MAP causes in humans.

Whilst accepting that this is an iconoclastic viewpoint, it is no longer out of the mainstream of academic perspective. A recent editorial in the NEJM stated that:

"It is tempting to speculate that these common genetic signatures support, albeit indirectly, the proposal that a proportion of Crohn’s disease cases may have a mycobacterial cause." 1

This comment in the NEJM are consequent to data showing a commonality in genetic defects in multibacillary leprosy and Crohn’s disease. 2 Additionally, there is parallism in the host immune response between multibacillary and paucibacillary leprosy with perforating and non-perforating Crohn's disease.3

There are important implications of these observations to this manuscript.

It is my opinion that there are two major reasons that the zoonosis of MAP has been missed.

First, in humans, MAP exists in the cell wall deficient from and therefore will not
be detected by the 1882/3 mycobacterial cell wall staining methods of Ziehl 4 and Neelsen. 5

The authors of the manuscript under review cite, but apparently have not understood, the significance of one of Ramon Juste’s manuscripts 6 (their reference # 36.) Either that is because the paper is incomprehensible, they have not read it or they did read it but did not understand it.

I hope that the first explanation is not the reason, as I wrote the Juste paper. Ramon contacted me with his unexpected observation of the prevalence of MAP DNA in the BLOOD of controls and patients with IBD. The published results in 6 are very similar to those found for MAP DNA in the TISSUE of the presented in this manuscript.

The clue to how I suggest a further analysis of data may prove valuable is found in final segment of Table 3 of this manuscript (Anti-inflammatory therapy: AIT) I previously suggested to Ramon that these analyses be expanded. Similarly I now suggest that these authors should also expand this medication analysis.

This is because the authors have ignored a new body of literature about IBD. I have suggested that, unknowingly, the medical profession has been treating MAP since Svartz introduced 5-ASA in 1942.7

There are now un-refuted data showing that agents referred to as “antiinflammatories” 8, “immune-modulators” 9-12 and “immunosuppressants” 13 all cause dose dependent inhibition of MAP in culture. In the Just manuscript, we were able to show that concurrent OR PRIOR treatment with these agents decreased or ELIMINATED the prevalence of MAP DNA in blood (depending on which therapeutic agents were used.)

If the authors can retrieve the detailed pharmaceutical history of the patients that they have studied, they may find compelling data. Additionally, to my knowledge this will be the first time that such a correlation has been made with TISSUE, rather than as Ramon did, with blood 6.

Finally, please remember that M. leprae has NEVER been cultured 14 and thus Koch’s postulates cannot be met for M. leprae and leprosy.15 MAP culture is not routinely possible in humans, as vanishingly few laboratories are able to culture MAP from humans.16,17 Additionally, when MAP is successfully cultured from humans, as much as 18 months may be necessary for the isolated bacterium to manifest a cell wall.16

Specific Suggestions:

#1.) The manuscript lacks a logical progression. What is mentioned first in the Methods section is not mentioned first in the Results or the Discussion. The authors should rearrange their manuscript so there is a consistent sequence throughout.

#2.) Just because you performed a lot of experiments, does NOT mean that you have to publish everything. For example, you present NO Results on the MAP culture component of the study. Accordingly, you should delete the Culture section from Methods.
#3.) Perform an analysis of the medications (concurrent and historical if possible) prescribed to your IBD cohort. You may find interesting data and (to my knowledge) for the first time show a detection effect of any antiMAP medications in TISSUE from an IBD cohort.

#4.) The authors have the possibility of presenting a MAP DNA/RNA comparison from IBD tissue.

As the authors state, there are differences in the detection, or inability to detect MAP DNA in IBD. Data from my laboratory found MAP RNA in all patients with UC and CD. 18 Subsequently, in unpublished data, we did NOT find MAP DNA in the same specimens in which we find MAP RNA. I have suggested technical reasons why this may be so. 15 To my knowledge, no other investigator has looked for MAP RNA.

Since the authors already have isolated RNA from the specimens that they studied for MAP DNA, they can also look for MAP RNA (REMEMBER you have to use Random Primers, NOT oligo dT primers as prokaryotic RNA does not have a poly A tail!)

#5.) MMP data

When I first reviewed the Juste data, I deleted all of his immunological data that he subsequently published 19 (Your Ref # 47.) To my mind it only made sense to perform such an analysis on his data when we had clarified the status in relationship to unanticipated antiMAP therapies 8-13 (see above.) However, Ramon felt that his analysis was sufficient and went ahead without considering a reanalysis (I declined to review the manuscript.)

You find no relevant pattern in your MMP data.

I suggest that you perform the analyses that I have outlined (#3 & 4 above.) If you have a story, tell it. THEN in light of those data reanalyze your MMP data and see if you THEN get a pattern that tells a story.

HOWEVER: IF YOU DO FIND COMPELLING MAP DNA/RNA data when analyzed from the antiMAP activity, I suggest that for the sake of clarity that you DO NOT PUBLISH in the same manuscript. The story will get lost and yet another paper that confuses, rather than illuminates, will be added to the miasma that is the MAP/ IBD conundrum.

#6.) Mention that amongst the most important insights that may be found in understanding the susceptibility to a MAP infection are the presence or absence of genetic diseases associated with CD. These include NOD2 20,21 and Nramp1 22 amongst others.23 Such evaluations were not performed in this study. In my opinion, in future, the presence or absence of such mutations will need to be identified in order to better understand the response, or lack of a response, by the host to a MAP infection.

The authors should comment on the need to identify genetic defects in future studies.

Overall, this was a well-performed study. In my opinion, the results do not confirm the hypotheses tested because of the lack of understanding of the
antiMAP activity of various medications.

Good luck.

Robert Greenstein

Citations:


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 have submitted Provisional patents that are the consequence of my publications
since 2005. The most relevant publications have been cited in my review. Patents applied for include a MAP vaccine patent, a MAP detection in blood use patent and a potential antiMAP medication patent. These applications have been funded out of pocket. There is no other outside financial support.