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: 23 December 2010

Reviewer: Paul Scully

Reviewer’s report:

This well written manuscript by Rath et al. studied MAP DNA in IBD patients and its relationship with MMP expression. The topic is relevant to gastrointestinal pathophysiology. The methods employed in the article are clear and well described. They are fully appropriate and are of a similar nature to other publications in this area.

Major Compulsory Revisions

1: A large amount of the introduction and discussion hypothesise that MAP might represent a causative agent for CD. However, the sample number of 14 CD patients is a very small number to be drawing any conclusions from. Is it possible to increase this number? A similar number to the UC cohort would have greater scope to potentially detect MAP DNA and so may more fully represent the prevalence of MAP in CD. In addition, it would then allow for a comparison with regard to MMP expression. MMP analysis on a greater number of CD patients may show a different response with MAP than UC as has been shown with regard to cytokine production (Ren et al, (2008) J Gastroenterology and Hepat, 310-314; Clancy et al, (2007) Digest Liv Dis, 39, 445-451; Sibartie et al, (2010) Inflamm Bowel Dis, 16, 296-304). The paper would be of a much greater relevance if it was possible to include a higher number of CD patients.

2: With regard to the patients recruited I can see the appropriateness involved in obtaining subjects from German and Norwegian populations, however, this raises a number of issues. Firstly, although the increase in MAP detection in the German cohort was noted as non-significant, the percentages detected at 29% for Germany and 14% for Norway does raise the possibility that with a higher number of subjects this could be of significant difference. Studies by Collins et al. (J. Clinical Micro, (2000), 4373-4381) have suggested that differences between a Danish and US population may be due to differences in BCG vaccination status and whether or not it is a rural population that is sampled. Are the German and Norwegian populations similar in this regard? Also, the controls are only taken from the German population and so it could be said that this study is not properly controlled with any Norwegian non-IBD subjects. Is it possible to include a Norwegian cohort as part of this control group?

3: With regard to MMP expression the method used to compare inflamed areas with non-inflamed areas to allow for interpersonal variation in expression is
merited, however, it does not allow for any indication of MMP expression within the control subjects. Would it be possible to do a similar normalization with the 2 control biopsy samples, albeit both samples are non-inflamed in order to get some indication of the affect of MAP positivity on MMP expression in the control cohort? If this was possible it could add greatly to the value of the study and also give an insight into MAP in non-IBD subjects.

4: As part of the methodology only the German patients were cultured for MAP investigation. Why was it not possible to conduct a similar design for the Norwegian samples? Also, with the high DNA positivity obtained would it be expected that some of the cultured samples be positive even with regard to the difficulties of culturing MAP? Was the method validated for culture and was any positive control included to ensure that the non-culture of MAP was not a methodology issue? Was there consideration to culturing subject stool samples in addition to aid detection, due to small size of biopsy used for culture.

5: In the discussion paragraph 4 a number of previous studies are mentioned that differ in their ability to detect MAP in CD patients. The methodologies and samples analysed are different for some of these studies as some analyse blood and others intestinal biopsy samples. Would it therefore be possible to expand this paragraph to briefly highlight how these studies differ?

6: With regard to the MMPs included in the study was there a specific reason to include the ones used (MMP-1, 7, 13, 19, 28) or was it part of a larger screening experiment? A number of publications have implicated MMP-2 and MMP-9 (including ref. 16) as playing a role in IBD so was there a reason for non-inclusion of these MMPs?

7: At the end of paragraph 2 in the background it is said “although frequently isolated from mucosal tissues of patients with CD, no cellular response and pathogenic mechanism has been identified so far” Is this correct? Studies such as from Clancy et al, (Digest Liv Dis (2007) 39, 445-451) has implicated increased TNF secretion as potentially part of a pathogenic mechanism.

8: As part of the discussion and conclusion the frequent detection within the normal grouping is potentially attributed to wide environmental distribution of MAP. If this was the case should this be reflected in a higher number of CD patients being positive for MAP?

Minor Essential Revisions

1: Methods: Patient and biopsy samples paragraph 2: Each biopsy sample from German patients (n=42) for MAP cultural investigation was placed in a 1.5ml sterile….” Reworded to aid clarity.

2: In the methods part under the “RNA purification, cDNA synthesis and RT-PCR for MMP analysis” section, 2 kits are mentioned, cDNA synthesis kit (Roche) and Omniscript (Qiagen). To the best of my knowledge these are 2 kits that serve the purpose. Therefore, were 2 different kits used for this part of the methods or
should one of these not be included in the text?

3: In the methods part under DNA extraction and PCR analysis for MAP detection, paragraph 1, it says that DNA was extracted using a modified protocol of the DNeasy Blood and Tissue kit. What was the modification employed?

4: With regard to the statistical analysis, box and whisker diagrams and Mann-Whitney U tests are employed. I would presume this is due to the non-parametric nature of the data but would it be possible to include an explanation within the methodology as to why these statistical tests were chosen?

5: Methods: Statistical analysis paragraph: Spelling error in middle of paragraph “lof” instead of “of”.

6: Methods: Statistical analysis paragraph: Spelling error at end of paragraph, should be “Mann-Whitney” instead of “Man-Whitney”

7: Discussion: Paragraph 1: Spelling error in middle or paragraph “specific” should replace “specificity approach”.

8: Discussion: paragraph 2: “the” should be removed from the sentence “…mandatory for diagnostic PCR applications to exclude the false negative results”

9: Discussion: Paragraph 4: “in” should be removed from the sentence “Within our study, we detected MAP DNA in only a low percentage of in CD patients”

10: Discussion: Paragraph 8: “result” should be used instead of “resultant” in the sentence “…it has been speculated that the frequent detection of MAP in healthy controls is a resultant of the wide distribution…..”

11: Figure 2B: The “e” is missing at the end of “negative” and “positive”

12: Figure 4: is mislabeled as “Figure 3”.

Discretionary Revisions

1: Background: Paragraph 3: “Furthermore” could be used to replace “Further, first” to aid legibility at end of paragraph.

2: Methods: Culture of biopsy specimens for MAP detection paragraph: “After incubation at 37°C for 12 and 52 weeks” Rewritten as only these 2 time points were used to the best of my knowledge

3: Methods: Culture of biopsy specimens for MAP detection paragraph: “ANV (Ampothericin B, Nalidixic acid, Vancomycin)” abbreviation with no explanation

4: Methods: DNA extraction and PCR analysis for MAP detection paragraph 1: “The DNA of the biopsy samples and MGIT cultures at 12 and 52 weeks was extracted using a modified protocol…” Rewritten to reflect time points used and deletion of “by” from the sentence.
Methods: DNA extraction and PCR analysis for MAP detection paragraph 2: “MAP specific genes F57 and…” Capital “F”.

For the molecular detection of MAP, two PCR systems were set up in parallel, a triplex real-time PCR targeting MAP specific genes F57 and ISMav2 and an internal amplification control (IAC), and a nested PCR.” Rewritten to aid legibility.

Briefly, the 50µl PCR mixture for the triplex real time PCR assay consisted of….” Rewritten to aid legibility.

Results: MMP expression in UC patients with and without presence of MAP DNA paragraph 2: “……MMP regulation in inflamed mucosa expressed as a multiple of basal expression…..” Rewritten to aid clarity.

Results: Detection of MAP DNA in IBD patients and controls paragraph: “None of the MGIT samples were positive for the cultural investigation.” Rewritten.

Discussion: Paragraph 5: “instead” should be removed from the end of the first sentence of the paragraph “….MAP DNA in patients with UC (20%) and controls (33%) instead”’

Discussion: Paragraph 4: “So far, a number of studies have reported more frequent detection of MAP DNA…..” Rewritten to aid legibility.

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.