Reviewer’s report

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

Version: 2 Date: 11 February 2011

Reviewer: Paul Scully

Reviewer’s report:

With regard to the resubmitted article I feel that the subgroup analysis and treatment analysis adds to the paper itself but I still have issues regarding a number of the major revisions mentioned previously. I have included the aforementioned compulsory revisions that I feel still need further clarification below.

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.

Author’s reply:

With this study we followed a dual intent: Our first aim was to determine the prevalence of MAP DNA in intestinal tissue of patients with IBD and healthy controls using sensitive and specific PCRs and MAP culture. Furthermore, it has been shown that PBMCs isolated from cattle infected with John’s disease
upregulate MMP-9 and TIMP upon stimulation with MAP (Coussens PM et al. Microb Pathog2004, 36:93-108; Coussens PM et al. Vet Immunol Immunopathol 2005, 105:221-234) and murine in vitro and in vivo studies provide evidence that MMP upregulation plays a pathogenic role in infections caused by pathogenic mycobacteria (Qiding-Jarbrink et al. Infect Immun 2001, 69:5661-5670). Based on this evidence, we further aimed to analyze whether IBD patients with intestinal MAP detection might exhibit a higher mucosal MMP expression than those without MAP infection. We agree with the reviewer that the inclusion of a higher number of CD would be beneficial as it would more fully represent the prevalence of intestinal MAP DNA in CD. However, as it unfortunately currently not possible to increase the number of CD patients we address to this limitation in the discussion of our manuscript in the following way: “As our cohort of patients with CD was relatively small, this result might represent an underestimation of the frequency of MAP DNA detection in CD patients. Thus, on the basis of our data, we cannot support nor exclude the hypothesis of an etiologic involvement of MAP in the pathogenesis of CD”. We also agree with the reviewer that MMP analysis on a greater number of CD patients might show a different response to MAP in CD than in UC. Nevertheless, given that we compared the MMP and TNF-# expression in UC patients with and without MAP infection, our approach should be sufficient to detect an in vivo regulation of the respective MMPs by MAP, although the magnitude of this regulation might differ from that observed in CD. Therefore, we think that the second intent of our study (to analyze whether MAP positive and MAP negative IBD patients exhibit a different intestinal MMP expression) can be addressed appropriately in our study, especially because of the fact that our UC cohort is quite large (n=49) and the number of UC patients with positive MAP (n=10) sufficient for statistical calculations.

REPLY:

Based on these numbers if the CD cohort is to be included as part of the paper I think that details of the power analysis undertaken will have to be included. The numbers of patients at 14 as far as I can tell is far too few to be making any comments at all and thus requires further detailing of statistical power analysis or consideration of not including at all seeing that the numbers cannot be increased. I agree with the comments on the UC cohort being of a large enough number but again for completeness further statistics would complement the numbers.

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 nonsignificant, 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?

Author’s reply:
We thank the reviewer for addressing this important point. German and
Norwegian patients were recruited from the endoscopic department of the
Universities of Giessen (Germany) and Stavanger (Norway) and its catchment
areas. Stavanger is the third largest city of Norway with approximately 190,000
people living in the Stavanger conurbation. Stavanger municipality has
approximately 120,000 inhabitants. Giessen municipality has approximately
80,000 inhabitants and the administrative county of Giessen enfolds 255,000
people. Therefore, all patients and controls from Germany and Norway recruited
for this study represent an urban population. Concerning the BCG vaccination,
the following can be said: In Norway, the BCG vaccination came into general use
in 1947 for the following populations: ALL 12–14-year-olds, children with
origins in high-incidence countries, children/adolescents with family history or
contact with TB case, children/adolescents before long-term travel to
high-incidence countries, health personnel at high risk of tuberculosis exposure
(Norwegian Institute of Public Health. Forebygging og kontroll av tuberkulose: en
veileder [Guidelines for prevention and control of tuberculosis]. Oslo, Norway:
Norwegian Institute of Public Health, 2002). BCG vaccination was compulsory
during the first decades, but has been voluntary and recommended since 1995.
The general vaccination coverage is calculated to be >90-95% (Infuso A, Falzon
D. European survey of BCG vaccination policies and surveillance in children,
Immunization coverage with BCG). The BCG vaccination is no longer generally
recommended since 2009. Therefore, one can assume that the great majority
of Norwegian patients in our study was BCG vaccinated. In Germany, BCG
vaccination was part of the generally recommended newborn vaccination regime
and vaccination coverage was calculated to be >90% (WHO). Since 1998, the
BCG vaccination is no longer generally recommended by the vaccination
committee (“Ständige Impfkommission”, STIKO) of the Robert-Koch-Institute. As
only adult patients were included in our study, it can be likewise assumed that
the great majority of the German IBD and control cohort was BCG vaccinated as
well. Given these considerations, we did not include a second control group from
Norway. Although we fully agree that the factors such as living area and BCG vaccination status are important to consider when analyzing the prevalence of MAP, we also believe that such an analysis is beyond the scope of our study as this would require considerably larger epidemiologic studies which assess a detailed individual history including, among others, factors such as housing/living situation, movements, travel history, number and origin of present and recent household members, present and previous contact to animals, detailed working history, eating and drinking habits.

REPLY:

With regard to this issue I have to ask the question as to why include a cohort from Norway at all? I understand the concept of allowing for locoregional differences but how can this be the case if the controls are all from one country only? This is a potential source of bias in the paper and does not allow the exclusion of locoregional differences. The majority of the patients are Norwegian (n = 42) with German patients (n=21) and German controls only (n=21). A more full explanation of the reasons for inclusion of these 2 populations should be included in the paper. This aspect is not discussed at all as far as I can see and I do not fully understand the basis and merit for the 2 populations. Could this be further expanded?

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.

Author’s reply:

For the MAP culture, biopsy samples taken during the colonoscopy were added to a reaction tube with 0.5 ml saline. To guarantee a fast processing and to avoid potential cross contamination, these biopsies were then immediately transferred to the Faculty of Veterinary Medicine located on the same medical campus. Unfortunately such an approach was not possible in Stavanger for the following reasons: MAP culture is not routinely possible in humans and cultivation of MAP from tissue, even from animals with Johne’s disease, is usually associated with many obstacles and therefore requires a certain level of expertise and
experience. This, in part, is because MAP is a fastidious and mycobactin-dependent bacterium with a generation time over 22 hours. The spheroplast form of MAP as reported in tissues of patients with CD presents even more obstacles to its isolation in a culture medium and in fact requires special media supplements with additives that support the difference in osmotic pressure between the intra- and extracellular environment. Further, it may take up to 18 months for the bacterium to manifest a cell wall so that a long culture period is required. Due to the lack of institutions meeting the outlined criteria in Stavanger, we unfortunately had to restrict the MAP culture to our German cohort. Stool samples for MAP culture were not used for MAP culture as they would not allow overcoming the difficulties associated with culturing MAP in vitro.

REPLY:
Again this raises the issues as to why a Norwegian cohort was included so needs to be more fully addressed in the paper.

Minor Essential Revisions
Page 18: top of page: “(tissue vs. blood) is the relevant size….”
“is” should be included to aid legibility.
Page 20: third line from top. “and MMP-2” is repeated.

Discretionary Revisions
Page 21: “Apart from that” can be left out in the third last line.
Page 18: Middle paragraph “Further” should be “Furthermore” to aid clarity.

Level of interest: An article of importance in its field

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