

October 10th 2013

Dear Editor,

Re: Manuscript NO: 5296

Please find enclosed the edited manuscript in Word format (file name: 5296-review.doc).

Title: Increased susceptibility to *Trichuris muris* infection and exacerbation of colitis in Mdr1a^{-/-} mice

Authors: Bhardwaj, Ekta K. Else, Kathryn J. Rogan, Michael T., Warhurst, Geoffrey

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 5296

Response to Reviewer 00049305:

The reviewer raised three main points, which are addressed below with the revisions to the manuscript indicated.

1. The manuscript has been revised to incorporate the change in title as suggested by the reviewer to "Increased susceptibility to *Trichuris muris* infection and exacerbation of colitis in Mdr1a^{-/-} mice".
2. We have clarified that IDO and CXCL10 expression shown in Fig 3 is measured in isolated colonocytes. The Methods section has been revised to include the method for colonocyte isolation and gene expression analysis.
- 3, Figure 5. The reviewer asks whether we could provide data on Th17-associated cytokines in addition to the Th1 and Th2 cytokines already shown. We agree with the reviewer that given the evidence that Th17 family cytokines play an important role IBD, this would be an interesting subject for future studies in this model. Unfortunately, we have no samples remaining with which to run additional analyses and would need to set up experiments with a new batch of animals to provide this information, which we would not be able to do within a reasonable time-frame. We have raised the issue of IL-17 in terms of future studies in the Discussion of the revised manuscript citing a recent study (Fasnacht et al Eur. J. Immunol. 2009. 39: 2173–2183) that showed parallel increases in IL-17 and IFN γ in response to *T. muris* infection.

Response to Reviewer 00049305:

The reviewer raised two minor points which are addressed below with the revisions to the manuscript indicated.

1. The manuscript has been revised to show the total number of animals used in the study (1st Paragraph – Materials and Methods). The number of animals associated with each Figure is shown in the Figure legends
2. Regarding the reviewer's comments on representation of ulceration in Figure 4. We believe that there is evidence of discontinuity in the epithelium in Figure 4D indicative of ulceration. However, in response to the reviewer's comments we have slightly modified the description of this Figure in the revised manuscript to highlight the evidence of crypt abscess rather than ulceration. We do however feel that Figures 4 D and 4E demonstrates clearly the severe pathology of the mdr1a^{-/-} mice following *T. muris* infection.

Response to Reviewer 00033708:

The reviewer raised a number of points which are addressed below with revisions to the manuscript where appropriate indicated.

Specific comments:

1. One of the main issues raised by the reviewer is that “the purpose of the study was to investigate how *T. suis* can ameliorate human inflammatory bowel disease, *T. muris* worsened the disease in *Mdr1a*^{-/-} mice. Therefore, this model was not suitable for this purpose”. The reviewer has misunderstood the purpose of our study. The aim of our study was to investigate the interaction of *Trichuris muris* with a mouse model of genetic susceptibility to inflammatory bowel disease (IBD). We had no preconceptions as to how the worm might influence IBD development if at all. The question was of particular interest because there remains uncertainty about the effects of helminths in IBD susceptible hosts with studies showing both amelioration and exacerbation of disease. The novel aspect of our study was that there is little information on the effects of *Trichuris* in these models with only a single published study in *IL-10*^{-/-} (a different model) where the precise contribution of the worm infection to the inflammation that develops spontaneously in this model was unclear. This study is the first to show that an IBD-susceptible host becomes more susceptible to *Trichuris* infection and, furthermore that infection is associated with a dramatic acceleration of colitis.
- 2 and 3. The reviewer suggests that we have not proven conclusively that a raised Th1 environment is the reason for the accelerated infection by *T. muris*. We believe that the indirect evidence in support of Th1 as the main driver of increased infection is persuasive in view of the evidence of increased IFN-dependent gene expression shown here and in a related study (Reference 11) in *Mdr1a*^{-/-} mice and previously published data from a member of our group showing that a similar profile of IFN-dependent gene expression is associated with susceptibility to *T. muris* infection (Reference 12). However, we agree with the reviewer that the current evidence does not provide conclusive proof and indeed acknowledge in the Discussion that further work will be needed to prove the primary role of Th1. However, while we intend to address this in future studies we feel that the current observations are of sufficient interest and topicality in light of the continuing uncertainty regarding helminth effects in IBD. We have also addressed the issue of relevance to man and acknowledge that it is difficult to determine at this stage. Nevertheless, as we point out in the Discussion, the current study raises interesting questions about a possible link between worm persistence and exacerbation of IBD. This is of relevance to human therapy since under normal circumstances *T. suis* does not appear to persist in human gut which may explain the ameliorating effects. However, our findings suggest that care should be taken in assessing the genetic susceptibility of the host because this could potentially be associated with increased worm persistence.
4. The reviewer asks about serum IgE and mucosal IgA responses. These parameters were not measured in this study; however serum levels of parasite specific antibody (IgG1 and IgG2) which are accepted measures of the immune response to worm infection are shown in Figure 2.
5. We did not systematically monitor macroscopic markers of colitis. We preferred to use histological analysis using an accepted disease scoring system which we believe provides a more reliable indicator of disease progression.

Minor points:

1. We thank the reviewer for pointing out this error. In the revised manuscript the symbols relating to the different groups are now correct
2. Figure 7: The green staining is epithelial cells stained for Cytokeratin – the legend has been revised to include this
3. Page 2 of Discussion - The additional period has been deleted 3 as requested in the revised manuscript.

The changes to the manuscript requested by the Editor including provision of PMID and DOI

information for references and providing graphs as decomposable figures have been made. Decomposable figures are provided separately as Powerpoint files.

Thank you for considering our revised manuscript for publication in the *World Journal of Gastroenterology*.

Sincerely yours,

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