

October, 5th 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5614-review.doc).

Title: Enteric bacterial proteases in inflammatory bowel disease- pathophysiology and clinical implications

Author: Ian M Carroll, Nitsan Maharshak

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 5614

The manuscript has been improved according to the suggestions of reviewers:

1. Format has been updated
2. Typesetting were corrected, the title was modified, the abstract was modified to have more than 200 words, a short summary has been added, the table and figure were moved to the end of the manuscript.
3. Revision has been made according to the suggestions of the reviewers.

(1) Reviewer 1:

1. In the first paragraph of introduction, the authors pointed out that it is detrimental to disrupt the epithelial barrier. It would be better if they could briefly discuss that protection of epithelial cells and restoration of epithelial barrier integrity alleviate IBD symptoms. Such a discussion will form a strong support about their late discussion on drug targets. such evidences can be seen in the paper as : Jiang GL, Nieves A, Im WB, Old DW, Dinh DT, Wheeler L. The prevention of colitis by E Prostanoid receptor 4 agonist through enhancement of epithelium survival and regeneration. J Pharmacol Exp Ther. 2007 Jan;320(1):22-8.

Response: We appreciate the suggestion and we have added the following text to the introduction (underlined):

"Additionally, disruption of the intestinal epithelial barrier by exposure of susceptible patients to NSAIDs (blockers of prostaglandins synthesis) is a known risk factor that can trigger the intestinal inflammation. Consistent with this observation, in animal studies, the use of a prostaglandin receptor agonist preserved the intestinal epithelial barrier

structure and function, maintained mucous secretion by goblet cells, and prevented the development of colitis^[8]."

2. On page 3, the last line, is that "IBS" or "IBD"? Please double check.

Response: This study was carried out on IBS patients as stated in the text. We cite this work since this the only example of an association between enteric bacterial taxa that was performed by using high throughput sequencing and proteolytic activity.

3. On page 13, it is hard for me to understand the following sentence, please modify:
"Spacifically, macfarlane...pancreatectomy.....that had a pancreas."

Response: We have modified the text to make the point clearer, as appears below:

"Specifically, Macfarlane and colleagues found that the proteolytic activity in the stool from a patient that had undergone a pancreatectomy was comparable to that of the protease activities in stools from individuals that had not undergone surgery to remove their pancreas. This indicates that a source other than the pancreas (i.e. enteric microbes) significantly contributes to the protease activity of the intestine."

(2) **Reviewer 2:**

This is a fairly comprehensive review of bacterial proteases in IBD, there are no major defects.

Response: We thank the reviewer for the favorable review.

(3) **Reviewer 3:**

Can this review be abbreviated somewhat so that the non expert can follow?

Response: We appreciate the comment, but since this subject is vast and not familiar to most readers, we have tried to be as comprehensive as possible.

(4) **Reviewer 4:**

1. The present paper aimed "to provide an overview of current studies that suggest potential mechanisms in which microbial proteases may play a role in the pathogenesis of IBD". It's a well-written paper very clear and consistent. However, I strongly suggest the authors to include a discussion section, where all the ideas could be put together and the data from different authors could be confronted.

Response: Thank you for the suggestion. The following section has been added to the discussion:

" However, it is not clear whether the proteolytic activity found in the gut lumen is exclusively of mammalian or bacterial origin. This is complicated by the fact that mammalian proteases, such as pancreatic digestive enzymes, are abundant in the gut lumen, and proteases secreted within the gut wall by leukocytes, such as neutrophils (cathepsin G) or mast cells (tryptase), "spill" into the inflamed gut. These factors may account for some of the discrepancies found between various studies investigating the origin of luminal proteolytic activity and the receptors they activate. Moreover, it is currently difficult to characterize luminal proteolytic activity, and while some research studies examine tryptic activity or gelatinase activity (each of which represents only a portion of the total luminal proteolytic activity) other studies have sought to characterize total luminal protease activity via functional assays and through inhibition of specific proteases activity. These challenges may also explain why it is not fully clear which PAR isotype mediates increased enteric permeability and inflammation. For example, PAR1 and PAR2 have been implicated in mediating enteric inflammation or permeability by bacterial proteases or mammalian proteases while activation of PAR4 can equally result in increased enteric permeability. It is not improbable to hypothesize that for increased intestinal permeability and colitis to occur there is a multi-factorial hit process that results in activation of multiple PARs simultaneously by different proteases."

2. Please add a reference in the end of the first paragraph of the introduction.

Response: A reference was added.

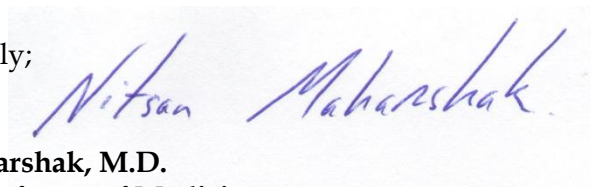
3. Please add a reference in the end of the first paragraph of page 4, before the aim.

Response: References were added.

4. On page 13, Figure 1, please correct the bacterial name to *P. gingivalis* (and not *P. gingaivalis*)

Response: Thank you- it has been corrected.

Most sincerely;



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