May 12, 2021

World Journal of Gastroenterology (WJG)
Re: “Antimicrobial Peptides and the Gut Microbiome in Inflammatory Bowel Disease”

We are very grateful for the opportunity to respond to the reviewers’ and editors’ critiques. Please see the revised manuscript with a point-by-point response to the reviewers’ comments.

We thank you for considering this manuscript for revision and look forward to your response.

Reviewer #1:
Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Minor revision
Specific Comments to Authors: The review by Gubatan et al. is a good manuscript but before this review is published I request that a paragraph on arachidonic acid metabolism in relation to the function and mechanisms of AMPs in the Pathogenesis of IBD be written. Indeed, the expression and activity of COX-2 is important in inflammatory bowel diseases as well as pro-inflammatory cytokines (IL-1, TNFa) that induce COX-2. Also in the "Donkey Milk Lysozyme" section, to my knowledge IL-13 is not a pro-inflammatory but an anti-inflammatory cytokine. This aspect needs to be corrected or better written.

RESPONSE: Thank you for this important feedback. We have added the following paragraph: “Arachidonic acid and its metabolism also play a role in the regulation of antimicrobial peptides in inflammatory bowel disease. Arachidonic metabolites such as leukotrienes and are elevated in both animal models of colitis and patients with IBD [86]. Leukotrienes have been shown to trigger release of human cathelicidin from neutrophils [87], whereas prostaglandins suppress cathelicidin in human macrophages [88]. In addition, cyclooxygenase-2 (COX-2), an enzyme that metabolizes arachidonic acid, is also induced in colonic epithelial cells in IBD [89]. Cox-2 selective inhibitors have been shown to inhibit production of human beta defensins but not cathelicidin [90].” The reviewer is correct that IL-13 is traditionally considered an anti-inflammatory cytokine, but studies have shown that IL-13 has pleiotropic effects including proinflammatory effects on intestinal epithelial cells resulting in apoptosis and epithelial barrier dysfunction in intestinal inflammation [94,95]. We have added this clarification in the manuscript: “The authors showed that 50% DML treatment brought cytokines, TNF-a and IL-13, a pleiotropic cytokine that has proinflammatory effects on intestinal epithelial cells resulting in apoptosis and epithelial barrier dysfunction in intestinal inflammation [94,95] back to basal levels similar to control mice.”

Reviewer #2:
Scientific Quality: Grade B (Very good)
Language Quality: Grade A (Priority publishing)
Conclusion: Accept (General priority)
Specific Comments to Authors: In this review/frontier article, the authors "discuss the function and mechanisms of AMP in the gastrointestinal tract, examine the interaction of AMP with the gut microbiome, explore the role of AMP in the pathogenesis of IBD, and review translational applications of AMP in patients with IBD." The authors make an elegant review of the literature, defining the biomarkers and even showing their clinical applicability. Calprotectin is the most widely used biomarker in clinical practice. Despite its routine use in clinical practice, there is still no well-established cut-off value for classifying patients in activity or remission of the disease. I suggest that the authors discuss the cut-off values of calprotectin in the differentiation between active disease versus remission, as well as whether there are established calprotectin values to determine therapeutic response to any medication, such as a drop of x% from the initial value, for example.

RESPONSE: Thank you for this recommendation. We have added the following "Cut-off values of fecal calprotectin to differentiate active disease versus remission in patients with IBD have been previously evaluated in a meta-analysis of 13 studies [108]: a cutoff value of 50 mg/g had a pooled sensitivity of 0.92 and specificity of 0.60 (0.52–0.67), a cutoff value of 100 mg/g had a pooled sensitivity of 0.84 and specificity of 0.66, a cutoff value of 250 mg/g had a pooled sensitivity of 0.80 (0.76–0.84) and specificity of 0.82 (0.77–0.86). Decreased levels of FC after therapy are associated with clinical, endoscopic and histological improvement with a normalization of FC (< 50 mg/g) signifying deeper remission [109]."

Reviewer #3:
Scientific Quality: Grade C (Good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Minor revision
Specific Comments to Authors: In the Frontier article: Antimicrobial Peptides and the Gut Microbiome in Inflammatory Bowel Disease, Gubatan et.al., present an overview of the major classes of AMPs in the Gastrointestinal tract (Table 1), results from functional studies of AMPs in preclinical models (Table 2) and studies of Biomarker applications of antimicrobial peptides in patients with IBD (Table 3). The review is nice and compact. However, the manuscript includes some structural problems and confusing descriptions.

In general, it would be useful to clarify where the data described were generated in animal models or cell lines, since it is often not obvious whether the authors are discussing human data or rather studies in e.g., rodents.

RESPONSE: Table 1 refers to the human gastrointestinal tract unless otherwise noted. We have revised our Table 2 to clarify which preclinical models used animal versus human cell culture models. Table 3 only includes human IBD studies.

Are there differences in distribution and regulation of AMPs in pre-clinical animal models compared to humans?

RESPONSE: Intestinal tissue expression of certain AMPs such as defensins, cathelicidin, and elafin appear comparable between animal models of IBD and tissue from patients with IBD. The included studies in Table 2 (preclinical IBD models) and Table 3 (human IBD) demonstrated that intestinal inflammation or disease activity were major drivers of changes in AMP expression.

In the main text, I think information in the sections "Antimicrobial peptides in the Gastrointestinal" tract and "Antimicrobial Peptides and Gut Microbiome" will appear less repetitive if the authors merge description of the different AMPs.

RESPONSE: Thank you for this good suggestion. We have merged the gut microbiome description for individual AMPs into one section "Gut Microbiome Effects of Different Antimicrobial Peptides" and removed any redundant descriptions repeated from the section "Antimicrobial peptides in the Gastrointestinal Tract."

The authors should avoid including many reviews by others as references (e.g., refs 5, 8, 14, 40, 41, 42, 43, 44, 46, 47, 48, 60, 68, …78, ++), or at least explain why they are included in the present review.

RESPONSE: We have removed the references above citing reviews and replaced them with appropriate primary studies.

Specific comments: Table 1: Antimicrobial peptides in the Gastrointestinal Tract. Description of tissue expression, location and cellular origin can be clearer. The precision level (i.e., Paneth cells, enterocytes of small and large intestine, colonic epithelial cells, Enterocytes, Epithelial Cells, Intestinal Epithelial Cells) appears somewhat random?

RESPONSE: We reported the data based on the level of precision of the individual studies. Some studies were specific with localization (e.g. Paneth cells) while other studies were more general (colonic epithelial cells). We have revised the vague description "enterocyte" with "intestinal epithelial cells."

What is the rationale for including studies of epithelia in human lung (ref 15), mast cells in skin (ref 17)?

RESPONSE: We have replaced Ref 15 with an appropriate study discussing the broad-spectrum antimicrobial activity of cathelicidin (Travis SM, Anderson NN, Forsyth WR, Espiritu C, Conway BD, Greenberg EP, McCray PB Jr, Lehrer RI, Welsh MJ, Tack BF. Bactericidal activity of mammalian cathelicidin-derived peptides. Infect Immun. 2000 May;68(5):2748-55. doi: 10.1128/iai.68.5.2748-2755.2000. PMID: 10768969; PMCID: PMC97484). Reference 17 was included to provide a study which showed expression of cathelicidin by mast cells. We have replaced Reference 17 with an appropriate study to

illustrate this point (Di Nardo A, Vitiello A, Gallo RL. Cutting edge: mast cell antimicrobial activity is mediated by expression of cathelicidin antimicrobial peptide. J Immunol. 2003 Mar 1;170(5):2274-8. doi: 10.4049/jimmunol.170.5.2274. PMID: 12594247).

Studies in animal models or cell lines like Caco2 and HT29 should be separated from studies in IBD-patients.

RESPONSE: We have organized Table 2 to illustrate preclinical models of IBD which included studies that used animal models together with human cell culture models. We have revised Table 2 to clarify preclinical IBD model "Preclinical Models (Animal, Human Cell Culture)." In the preclinical models of IBD table, there were very few studies with human cell lines to justify creating another table and thus kept the studies under the same Table 2 but clarified which studies included human cell lines in addition to animal models to improve clarity.

I also suggest including some references from human studies for all AMPs. Table 3: I would recommend that the authors consider including some recent publications like "Faecal Biomarkers in Inflammatory Bowel Diseases: Calprotectin Versus Lipocalin-2-a Comparative Study" PMID: 32556317

RESPONSE: We have added the reference above to Table 3.

Sincerely,

On behalf of co-authors:
John Gubatan, MD
Stephan Rogalla, MD, PhD