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Manuscript NO: 76320 MINIREVIEWS

Immunological Mechanisms of Fecal Microbiota Transplantation in recurrent *Clostridioides difficile* infection

Running title: Fecal Transplant & Immunological Mechanisms

Lucas F. Soveral, Gabriela G. Korczaguin, Pedro S Schmidt, Isabel S. Nunes, Camilo Fernandes, Carlos R. Zárate-Bladés

Answers to reviewers:

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: The authors performed an uptodate mini-review focusing on the immune mechanisms involved in the host's response to FMT when treating rCDI. The paper brings insights into the new immunological pathways contributing to restoration of the host's mucosal barrier after the acute inflammation caused by *C. difficile* colitis. The paper is well structured, easy to read and concise. I identified two minor issues related to citing of information (page 4, paragraph regarding the secondary dysbiosis lacks citation),

Answer: We are thankful for reviewer's comments. We have added the references as requested.

as well as a phrase that I would recommend to be reformulated (page 5, last paragraph, there is also a citation lacking, as well as the information that states *C. difficile* antibiotic resistance as the main cause for recurrent CDI?).

Answer: Thank you for the observation. We have reformulated the sentence as requested and included the references as needed.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade A (Priority publishing)

Conclusion: Major revision

Specific Comments to Authors: Soveral et al. minireviewed immunological mechanisms of FMT in rCDI. The title, "Immunological Mechanisms of Fecal Microbiota Transplantation in Recurrent *Clostridioides Difficile* Infection", may be more appropriate.

Answer: We liked the reviewer's suggestions and changed the title of the mini-review.

The history and pathophysiology of CDI, in paragraph "CLOSTRIDIoidES DIFFICILE INFECTION AND INTESTINAL DYSBIOSIS CORRECTION WITH FMT", may be needless in this minireview. They are beside the question.

Answer: We appreciate the reviewer's suggestion, but considering the opinions from the other two reviewers, we opted to keep the information presented since we consider that it is not long and it helps the reader, that might not be specialist in the field, to have a concise overview of the disease. We hope that the reviewer #2 could accept this justification.

Besides, I think, if possible, the relationship between immunological molecules and microbial metabolites should also be illustrated. Microbes and their metabolites are both significant in FMT.

Answer: Taking in consideration this important suggestion we have included in the text a discussion about bile acids metabolism as well as of short-chain fatty acids as they are two key types of molecules that have been already showed to have important participation in FMT against CDI.

The language is idiomatic and I recommend the paper to need major revision.

Answer: We have sent the new version of the article for a new round of English checking by a specialized company and we have uploaded to the journal's system the certificate of this service together with the files of our manuscript.

Reviewer #3:

Scientific Quality: Grade A (Excellent)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Specific Comments to Authors: Comments: This an updated mini-review focusing on FMT immune mechanism. It is integrative and helpful for understanding this topic. The structure and the language are good.

1. Main comment: There is one important point on the core difference of the methods of FMT (using bacteria or filtered matters) which essentially affects the immune pathways. This should be discussed clearly in the manuscript. The origins of the both reports were shown as below:

-Using filtrated matters: Ott SJ, et al. Efficacy of Sterile Fecal Filtrate Transfer for Treating Patients With Clostridium difficile Infection. Gastroenterology. 2017 Mar;152(4):799-811.e7.

-Using purified bacteria Zhang T, et al. Washed microbiota transplantation vs. manual fecal microbiota transplantation: clinical findings, animal studies and in vitro screening. Protein Cell. 2020 Apr;11(4):251-266.

This method as medical therapy has been used as expert recommended practice: Nanjing consensus on methodology of washed microbiota transplantation. Chin Med J (Engl). 2020 Oct 5;133(19):2330-2332. doi: 10.1097/CM9.0000000000000954.

Feuerstadt P, et al. SER-109, an Oral Microbiome Therapy for Recurrent Clostridioides difficile Infection. N Engl J Med. 2022 Jan 20;386(3):220-229.

This method as IND has been used in clinical trial with success.

Answer: We are very thankful to the reviewer and we have included a discussion about all these important aspects of FMT in rCDI, which we believe it has improved the scientific quality of our minireview.

2. Minor comment: In page 7: "The first attempts to gain knowledge to elucidate the immunological events elicited by FMT to successfully treat rCDI in 2017". This is an old question, though it is not enough clear. Therefore, it is impossible that this topic was studied in 2017. Researchers in the world have studied this for many years.

Answer: We have performed a correction to this sentence to improve clarity of the sense of our text.

Re-reviewer:

Scientific Quality: Grade D (Fair)

Language Quality: Grade B (Minor language polishing)

Conclusion: Rejection

Specific Comments to Authors: This minireview is supposed to summarize studies on the immune response to the treatment of FMT in rCDI. Although the content is novel and interesting, the present manuscript has some questions as followed: 1. In abstract, the background of FMT is introduced, while the relationship with rCDI or immunity is not fully elucidated. The applications of FMT in other diseases do not match with the title.

Answer: We have modified the abstract following the suggestion and the new version presents to the reader the main immunological data that will be discussed in the review.

2.The introduction that describes the functions of human microbiota and the questions of dysbiosis is off the point.

Answer: We have modified the introduction section eliminating both points mentioned by the reviewer.

3.Though manuscript lists many examples of trials to describe the immunoreactions, few of the internal points have been summarized. The manuscript is indeed interesting and promising, but there is not enough scientific construction and orderliness to publish in the WJG.

Answer: Taking in consideration this particularly important observation, we have included several improvements to our manuscript: 1) A new figure 1. This figure summarizes the FMT variations and key features that support its effectiveness. These data is discussed in section “Clostridioides difficile infection and intestinal dysbiosis correction with FMT” 2) Table 1. This table is new, and summarizes the most relevant findings of the articles discussed in our manuscript in section “Immunological effects associated with FMT efficacy to treat rCDI” 3) A paragraph that summarizes all the evidences reviewed in the article about the microbial dereived molecules with immunological properties, as well as the discoveries about the immune system components that are important for FMT efficacy. 4) Improvement of original Figure 1. This figure is now Figure 2 in the new version of the manuscript. This figure illustrates the immune response to CDI and the effects on the immune system after receiving FMT. We are thankful for reviewer’s comments and we believe that all the modifications included in the new version of our manuscript will be accordingly for publication in WJG.