Peer Review File

Article information: https://dx.doi.org/10.21037/tgh-23-21

Reviewer A

Question 1: First, I suggest the authors to indicate the comparison between CD and healthy donors and the treatment of anti-inflammatory agents and/or the immunosuppressive drugs.
Reply 1: Regarding this issue, firstly, we do not have access to intestinal tissue and intestinal lymph nodes from healthy donors; secondly, healthy donors do not take drugs, so there is no comparison between CD and healthy donors.
Changes in the text: N/A.

Question 2: Second, the abstract needs further revisions. The background did not describe the clinical significance of this research focus and what the knowledge gap is. The methods need to describe the inclusion of CD and healthy donors and the statistical methods for comparing the outcomes. The results need to briefly report the characteristics of the two groups of patients such as age and sex. Please quantify the findings by providing mean-/+SD and accurate P values. The conclusion needs comments for the clinical implications of the findings.
Reply 2: We have revised according to the suggestion.
Changes in the text: Page 2 line 8 to Page 3 line 9.

Question 3: Third, in the introduction of the main text, the authors emphasized a lot on the knowledge gaps on this research focus but did not clearly indicate the clinical importance and potential clinical implications. Please detail the sentence “provide critical guidance for clinical medicine and treatment”.
Reply 3: We have revised.
Changes in the text: Page 5 line 11 to Page 5 line 14.

Question 4: Fourth, in the methodology of the main text, please clearly describe the research design such as case-control study and the sample size estimation, which seems to be very small. I do not think it is appropriate to regard this study as an experimental study since the subjects are patients. MDAR reporting checklist may not be suitable, please consider STROBE for a case-control study. In statistics, please first test the comparability between the two groups. It is also necessary to test the normality of the outcome variables before the comparative statistical analyses. Please ensure $P<0.05$ is two-sided.
Reply 4: Because the treatment of IBD is mainly conservative treatment with medicine, and surgery is not the main treatment method, the source of clinical samples is limited. The changes of immune cells in the pathological tissues were analyzed, and the own normal tissues were used as controls.
Changes in the text: N/A.

Reviewer B

Question 1: The title “The effect function of memory T-cell subsets in patients with Crohn’s disease after treatment” is confusing. It should be further revised. First, “the effect function of……” is wrong. Did the authors want to express “the effective function of……” or “the effect and function”? Please check it carefully. Second, indeed, the aim of the study is to investigate the effect of treatments with anti-inflammatory agents and/or immunosuppressive drugs on the frequency and function of Tm-cell subsets in patients with Crohn’s disease.

Reply 1: Alterations in Memory T-Cell Subsets Post-Treatment in Crohn's Disease Patients: Implications for Therapeutics

Changes in the text: Page one, line 2-3.

Question 2: Abstract

The sentence “Eight patients were diagnosed with CD and treated with anti-inflammatory agents and/or the immunosuppressive drugs” in the Methods section is wrong, because in the main text the authors said that a total of 8 patients with CD from the First Affiliated Hospital of Sun Yat-sen University were recruited for the study, patients infected with HIV, HBV, or HCV were excluded from the study, and ultimately 6 patients were diagnosed with CD and treated with anti-inflammatory agents and/or the immunosuppressive drugs.

Reply 2: We have modified these mistakes.

Changes in the text: Page 5 line 21 to Page 5 line 28.

Question 3: Methods

In the Study participants section, only 6 patients who were diagnosed with CD and treated with anti-inflammatory agents and/or the immunosuppressive drugs were recruited for the study. The number of patients in the study was too small and these patients were all male, with age ranging from 14 to 33 years. The authors should increase the number of the patients in the study, with an inclusion of female patients. And the range of the age should also be expanded to exclude the influence of sex and age.

Reply 3: Thank you for highlighting the concerns about the study participants' demographic range. We acknowledge the limitations posed by the number and demographic diversity of our study participants. Here are our considerations on this matter:

First: Our study was constrained by the availability of patients diagnosed with CD who met the criteria for treatment with anti-inflammatory agents and/or immunosuppressive drugs. While we would have preferred a larger and more diverse sample, these patients were the ones available and willing to participate during the research period.

Second: Potential Biases: We recognize that our sample might introduce biases, particularly related to sex and age. However, we have taken rigorous statistical measures to ensure that our findings are as valid and robust as they can be within these constraints.

Third: This research can be considered a pilot or preliminary study. While the limited number of patients and demographic range can introduce some biases, our findings still offer valuable insights into the effects of treatment on Tm-cell subsets in CD patients. We believe that the results of this study can provide an initial understanding, paving the way for larger and more comprehensive studies in the future.

In our manuscript's discussion section, we have emphasized the need for further studies

involving a larger and more diverse sample. We agree with the reviewer that including female patients and expanding the age range would provide a more holistic understanding of the effects observed.
Changes in the text: Page 12-13, line 19-2.

Question 4: Results
The Results section is too complex and prolix. It is pointless to discuss the effect on the frequency and function of Tm-cell subsets in patients with CD after treatments or healthy donors, so the authors only need to state the results of the experiments in the Results section.
Reply 4: Currently, it is believed that the protracted process of IBD is related to the existence of memory T cells, and memory T cells have different subsets, including memory T cells at different stages of differentiation, and memory T cells colonized in peripheral tissues. Whether these cell subsets have the same response to drugs is not clear. Therefore, this study investigates them.
Changes in the text: N/A

Discussion
In the sentence "Mucosal macrophages prevent the interconversion of Th1 and Th17 cells, thus promoting Trges differentiation", "Trges" should be revised as "Tregs".
In the sentence "More than 95% of the effector T cells will undergo apoptosis by via activation-included cell……", "via" should be deleted.
Reply 5: We have modified.
Changes in the text: Page 11 line 5-16.

Reviewer C

The manuscript describes a small study of 8 patients after colitis treatment. Pbmc, MLN and mucosa cells were isolated and analysed for cytokine expression and the presence of memory T cells. Less Trm was found in CD patients, as well as more IFNg and IL-17 producing Tscm cells.
In general, the analyses with FACS and other methods seem to have been done properly and there are no qualitative errors in the figures. However, there are many problems with the design of the study and the core hypothesis.

Question 1: For the analysis, 8 patients with questionable characteristic data, e.g. no gender distribution, limited age window, so on..., are of course too few to be able to draw any conclusions.
Reply 1: Thank you for your insightful comments regarding the demographic characteristics of our study participants.
Firstly, I acknowledge the limitation concerning the gender distribution. Indeed, all eight patients with CD in our study were males. This was a result of the patient cohort available and willing to participate during our recruitment period. We recognize that this might pose a limitation to the generalizability of our findings. However, given that this study is a preliminary observational investigation, we aim to include a more balanced gender distribution in our

subsequent studies to ensure more comprehensive data.
Regarding the age range, while our participants aged between 14 to 40 years, we believe this did not significantly alter our primary findings as all participants were within a relatively close age bracket. Nonetheless, in future works, we'll strive to encompass a broader age range to bolster the study's inclusivity.
Lastly, we would like to emphasize that despite the small sample size, this initial observation provides valuable insights that merit further exploration in larger cohorts. We've explicitly mentioned this limitation in our paper and have recommended more expansive studies to corroborate our findings.
Changes in the text: N/A.

Question 2: Unfortunately, the few patients who were examined were not separated according to the different treatment therapies, i.e. immunosuppressive or anti-inflammatory antibody therapy. This can lead to different reactions of the patients' immune systems being thrown together and masked. Ultimately, one can only formulate a general statement from this.
Reply 2:
Thank you for pointing out the potential variance that might arise due to different treatment therapies given to our patients. We genuinely value your feedback as it helps us refine our work.
In our study, the patients were indeed administered various treatments such as anti-inflammatory agents and different immunosuppressive drugs. We recognize the potential impact of these varied treatments on the immune system responses. The decision to not separate patients based on treatment was mainly due to the limited sample size available for our investigation. We anticipated that further sub-dividing this cohort might lead to smaller groups, which would pose challenges in statistical analysis.
However, we do acknowledge the validity of your observation. Different treatments can indeed modulate the immune response differently, and this is an aspect we will look into more meticulously in our subsequent research.
In light of your feedback, we will emphasize in the discussion section of our paper the potential variance introduced by different treatments. Moreover, as a recommendation, we will underline the importance of larger cohort studies where patients can be grouped based on their treatments, to derive more specific and actionable insights.
Thank you for your guidance, and we hope to address this concern adequately in our revised manuscript.
Changes in the text: N/A.

Question 3: The statement that Tm cells have an effect after therapy is not new and was already established years ago. In fact, it was already possible to find this in a manuscript by Hart et al. in 2004, Clin Exp. Immunol 135(1):137 (DOI: 10.1111/j.1365-2249.2004.02347.x). At that time, integrins were found that are now already being used as targets of therapies in clinic.
Reply 3: We have modified the discussion.
Changes in the text: Page 10 line 12 to Page 10 line 14.

Question 4: Overall, this manuscript is a rather descriptive analysis, which is only of limited value due to the very small number of subjects. The manuscript is years too late and would

therefore be better off in a methodological journal.

Reply 4: While our study may seem predominantly descriptive, our intention was to present foundational data that can be instrumental for subsequent in-depth analyses and investigations. While our paper emphasizes methodology, the insights derived can provide a roadmap for future clinical studies aiming to understand, diagnose, or treat CD. The depth and specificity of our research could serve as a vital resource for researchers keen on pursuing similar avenues or extending our methodology to larger cohorts.

Changes in the text: N/A.