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Subrata Ghosh, AGAF, FCAHS, FRCP (C), FRCPC, FRCPE, MD, Full Professor  
Andrzej S Tarnawski, DSc, MD, PhD, Professor

Editors-in-chief, *World of Journal of Gastroenterology*.

Dear Professor Ghosh,

Dear Professor Tarnawski,

Upon invitation, we submitted our work entitled “Anti-TNF $\alpha$  therapy associates to Th17 immunological shift and significant microbial changes in dextran sodium sulphate colitis” (Invitation ID: 03253997).

This is a revised manuscript, and it has been modified according to the comments made by the Reviewers. All changes made in the revised manuscript are underlined, and we made a point by point answer to reviewers questions below. The language editing was performed by a non-native English speaker who has worked for several years in Birmingham (UK) at Birmingham Women’s and Children’s NHS Foundation Trust. We thank the Editors and the Reviewers for considering our findings interesting and for their insightful comments.

With this manuscript we aimed to evaluate, in colitic mice as well as healthy mice, intestinal immune system status and gut microbiota modulation induced by anti-TNF $\alpha$  therapy. Healthy

mice treated with anti-TNF $\alpha$  showed similar histological, microbial and immune features of untreated colitic mice, in particular a lymphomononuclear infiltrate both at V and XII day at hematoxylin&eosin staining, an increase of Th1 and Th17 at day XII, and finally increase of Enterococcaceae at day V, a decrease of Bacteroides and Clostridiaceae at day XII.

These findings are particularly relevant to understand the role that anti-TNF $\alpha$  modulation plays on gut microbiota (and vice-versa?) also in humans: this finding could be of major interest in order to give more lights on mechanisms of loss of response to anti-TNF $\alpha$ , mechanisms of immunological shift with towards other pathways, like Th-17 pathways, as well as novel potential therapeutic targets in IBD.

A more comprehensive approach including gut microbiota modulation, if clarified, could be assessed by dedicated studies on active gut microbiota modulation during biologic therapy in IBD. This paper has been sustained by FONDAZIONE IN RICERCA E MEDICINA ONLUS (Bologna, Italy) and authors do not have direct conflicts of interest. The work has been completely undertaken in Rome, at Fondazione Policlinico Universitario “A. Gemelli” and Università Cattolica del Sacro Cuore, accounting for the collaboration of several research groups. We hope our work could encounter the interest of your journal.

Warm regards,

Franco Scaldaferri, MD, PhD, on behalf of all authors.



**Reviewer's code:** 01557050

**SPECIFIC COMMENTS TO AUTHORS**

1) General comments Dr. Petito and Lopetuso, et al. investigated 'Anti-TNF $\alpha$  therapy associates to Th17 immunological shift and significant microbial changes in dextran sodium sulphate colitis'. The article is informative and well-presented. The reviewer has some comments. Comments 1) The reviewer could not understand well about T0 and T2. Please describe the definition of T0 and T2 in Methods. **Done**

Page 7: "The results were divided between T0 and T2, where T0 represented the conditions before any treatment, and T2 represented the changes that appeared after 5 or 12 days of administration of DSS and anti-TNF $\alpha$ , alone or in association. The exact number of days was specified in the figures."

**Reviewer's code:** 04091933

**SPECIFIC COMMENTS TO AUTHORS**

This is indeed a high-quality study that demonstrates the viability of murine DSS-induced colitis model for studying the effects of anti-TNF- $\alpha$  (Infliximab, IFX) on the immune system and gut microbiota. The authors have previously shown that IFX showed a good affinity both for human-TNF- $\alpha$  and murine-TNF- $\alpha$ , and it ameliorated the severity of DSS colitis in mice [Lopetuso L.R. et al., 2013]. The new study clearly demonstrated that the anti-inflammatory effect of IFX is associated with an increase in Th17 pathway, along with gut microbiota alteration. An important clinical consequence, if it will be confirmed in humans, is the identification of a potential dysbiotic effect of IFX. This anti-TNF- $\alpha$ -induced dysbiosis should be taken into consideration, and not only in non-responders. Perhaps, some IBD patients with dysbiosis on anti-TNF- $\alpha$  will require therapeutic modulation of gut microbiota.

Furthermore, anti-TNF- $\alpha$ -induced shift toward Th17 pathway could be considered when deciding to switch/change therapy in non-responders. The limitations are described in detail by the authors and do not affect the quality of the article. In my opinion, the results of this study will lead to a significant breakthrough in future clinically important studies of the interaction between biological therapy, the immune system and gut microbiota.

In turn, mouse models will help develop effective methods for the therapeutic correction of dysbiotic microbiota in IBD.

Minor language polishing. **Done**