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PEER-REVIEW REPORT

Name of journal: *World Journal of Gastroenterology*

Manuscript NO: 76404

Title: Involvement of toll-like receptor 5 on mouse model of colonic hypersensitivity induced by neonatal maternal separation

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 04105454
Position: Editorial Board
Academic degree: MD
Professional title: Professor
Reviewer's Country/Territory: Egypt
Author's Country/Territory: France

Manuscript submission date: 2022-03-15

Reviewer chosen by: AI Technique

Reviewer accepted review: 2022-04-22 05:34

Reviewer performed review: 2022-04-22 05:43

Review time: 1 Hour

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [Y] Accept (General priority) [ ] Minor revision [ ] Major revision [ ] Rejection
Re-review	[ ] Yes [Y] No

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

It is well written paper with accepted abstract ,introduction ,method and discussion results section need simplified tables to present the results it would be a good advantage

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Reviewer's code: 00055107
Position: Editorial Board
Academic degree: PhD
Professional title: Full Professor
Reviewer's Country/Territory: Spain
Author's Country/Territory: France

Manuscript submission date: 2022-03-15

Reviewer chosen by: AI Technique

Reviewer accepted review: 2022-04-20 16:22

Reviewer performed review: 2022-04-25 16:06

Review time: 4 Days and 23 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

SPECIFIC COMMENTS TO THE AUTHOR GENERAL COMMENTS: Authors study the role of microbiota and TLR receptors on an animal model of colonic hypersensitivity. They found that neonatal maternal separation (NMS) in mice induced fecal microbiota dysbiosis characterized by a general decrease in bacterial species richness although in some genera the abundance of bacteria was increased. In addition, NMS induced overexpression of TLR5 in colonocytes whereas the TLR5 agonist flagellin mimic colonic hypersensitivity evoked by NMS. This paper presents interesting data, however I consider that it should be improved at several points shown below. SPECIFIC COMMENTS: Materials and Methods: The work is focused on the study of the pathophysiological pathways involved in the development of colonic hypersensitivity. Authors use the colorectal distension test with a pressure transducer catheter coupled to a balloon, an electronic barostat and a system to acquire the signal from the transducer. However, no information is given on the quantification of this signal to show the intracolonic pressure variation (IPV) and to obtain the area under the curve (AUC). In addition, authors should provide information on how these two parameters can reflect an increase in the colonic hypersensitivity. What numerical criteria have been followed to include NMS animals in each of the following categories: NMS NS and NMS? Statistical test used to check if data follow a normal distribution should be given. Results: In general, the Results section is hard to read due to the large amount of numerical data that are written in the text. I suggest to remove all data about means ± SEM in the text of the Results section as well as the statistical significance because they are already shown in the figures. Why data showing the relative abundance of bacteria

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belonging to the phylum Bacteroidetes and Firmicutes are not given and only is expressed as "data not shown"? I consider that it is a relevant result and indeed authors used it in the Discussion section. In P12 lines 255-266: This paragraph is confusing: 1) This statement is not correct: "NMS S mice were characterized by a decreased abundance of bacteria of the genera Bacteroides …, Barnesiella … and Allobaculum … compared to control NH mice". In fact, as can be seen in Fig. 3C, Bacteroides are less abundant in NMS S mice when compared with NMS NS. However, it is similar to NH mice. Thus, significance of NMS S observed in figure is "$$$" and not "***". 2) In the same paragraph authors stated: "whereas the relative abundances of Clostridium … and Lachnoclostridium … were increased in these NMS animals with CHS". This is not true because Fig 3C shows that the relative abundance of Clostridium in NMS S animals is similar to NH animals. Only NMS S animals shows fewer abundance compared to NMS NS animals ($). This point is very important because this result is also misunderstood in the Discussion section (P15 lines 342-343) Thus, I suggest rewriting this paragraph in Results and Discussion sections with more precise comparisons. Abstract: The Results section of the abstract is too short. Author should explain more in deep their main results, particularly those of fecal microbiota diversity. If it is necessary, Material and methods section can be shortened. Figures: The text of the figure legends is too large. I suggest deleting the statistical test used in the experiments. In Fig. 1B, the term "20" (minutes) that appear just to the right of "Intrarectal instillation" I think it should be replaced with "30" Legend of Fig. 2: 1) I suggest changing the statement: "* or $ p<0,05; ** or $$ p<0,01; $$$=$ p<0,001, respectively vs. NH or NMS NS groups" by this one: "* p<0,05 and ** p<0,01 vs. NH group; and $ p<0,05, $$ p<0,01 and $$$ p<0,001 vs. NMS NS group" 2) I suggest inserting "and FIT-Dextran" in this statement: "For AUC and FIT-Dextran, each dot represents one mouse and red lines represent means" Legend of Fig. 3: Authors stated "For alpha-diversity analysis, dots represent means and

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error bars represent SEM". However, no error bars are represented in this figure. Figures 4A and 4C: To avoid confusion, please use the symbol "$" instead "*" in comparisons between NMS S and NMS NS groups, and include it in the figure legend. Legend of Fig. 5: Remove the sentence: "Values are expressed as means and error bars represent SEM", because it is repeated in the legend. I suggest replacing the statement of statistical significance with a shorter one: "*p<0,05, **p<0.01 and ***p<0.001 respect to Baseline" In the Y axis from Figs, 2A, 5A and Suppl 1A: "Intracolonic Variation Pressure" should be replaced with "Intracolonic Pressure Variation" In the Y axis from Figs, 2C, 3A, and Suppl 1A: "Dextran-FITC" should be replaced with "FITC-Dextran" The number of animals used in the experiments ("n=…") can be removed from the Figs, 2A, 5A and Suppl 1A In Fig. Suppl 1A the number of animals used for NMS NS is different in the figure ("n=7") and in the legend of this figure ("n=6"). Figure legends should be self-explanatory as possible. Some abbreviations should be included in full: "NMS" in Fig. 1, "OTU" in Fig. 3, "Flagellin (FliC)" instead "FliC" in Figs. 1, 4 and 5. Minor comments: P6 Line 111: Replace "LPS" with "lipopolysaccharide (LPS)" and remove other "lipopolysaccharide (LPS)" that can be found thereafter in the text. P9 line 177: Replace "OTU" with "Operational Taxonomic Units (OTU)" P10 line 205 and P12 line 270: Replace "Flagellin (FliC)" with "FliC" P10 line 210: Replace "E. coli" with "Escherichia coli" P10 line 222: Replace "Quantitative Insights into Microbial Ecology pipeline (QIIME)" with "QIIME" P11 line 235: Replace "Dextran-FITC" with "FITC-Dextran" P12 line 259: "Allobaculum" should be written in italic

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RE-REVIEW REPORT OF REVISED MANUSCRIPT

Name of journal: *World Journal of Gastroenterology*

Manuscript NO: 76404

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Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 00055107
Position: Editorial Board
Academic degree: PhD
Professional title: Full Professor
Reviewer's Country/Territory: Spain
Author's Country/Territory: France

Manuscript submission date: 2022-03-15

Reviewer chosen by: Han Zhang

Reviewer accepted review: 2022-06-13 16:50

Reviewer performed review: 2022-06-14 17:58

Review time: 1 Day and 1 Hour

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Peer-reviewer	Peer-Review: [Y] Anonymous [ ] Onymous

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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

SPECIFIC COMMENTS TO THE AUTHOR Authors have answered and included in the manuscript satisfactorily all the main questions. However, several minor questions that were included in my referee's comments sent in April have not been answered. They are some minor concerns in the text, and especially in the figures and figure legends. Then, I consider that this paper could be improved at several points shown below before it will be published. Figures and Figure legends: Fig. 3D: Error bars representing SEM are absent, whereas they appear in the Fig 3C. Figures 4A and 4C: To avoid confusion, please use the symbol "$" instead "*" in comparisons between NMS S and NMS NS groups, and include this symbol in the figure legend. In Figs 2A, 5A and Suppl 1A, in the Y axis, the term "Intracolonic Variation Pressure" should be replaced with "Intracolonic Pressure Variation". In Figs 2C and Suppl 1A, in the Y axis, the term "Dextran-FITC" should be replaced with "FITC-Dextran". In Figs, 2A, 5A and Suppl 1A, the number of animals used in the experiments ("n=…") can be removed because they are in the Figure legends. In Fig. Suppl 1A the number of animals used for NMS NS is different in the figure ("n=7") and in the legend of this figure ("n=6"). Legend of Fig. 2: I suggest changing the statement: "* or $ p<0,05; ** or $$ p<0,01; $$$=$ p<0,001, respectively vs. NH or NMS NS groups" by this one: "* p<0,05 and ** p<0,01 vs. NH group; and $ p<0,05, $$ p<0,01 and $$$ p<0,001 vs. NMS NS group" Furthermore, I suggest inserting "and FIT-Dextran" in the last statement: "For AUC and FIT-Dextran, each dot represents one mouse and red lines represent means" Legend of Fig. 5: Remove the sentence: "Values are expressed as means and error bars represent SEM", because it is repeated later in the same legend: "dots represent means and error bars represent SEM". In addition, I suggest replacing the statement of statistical

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significance with a shorter one: "*p<0,05, **p<0.01 and ***p<0.001 respect to Baseline". Figure legends should be self-explanatory as possible. Thus, some abbreviations should be included in full: "NMS" (in Fig. 1), "OTU" (in Fig. 3) and "FliC" (in Figs. 1, 4 and 5). Minor comments in the text: The scientific name of one species should be in full the first time that it appears in a text, thus replace "E. coli" with "Escherichia coli" in the Materials and Methods section "Quantitative Insights into Microbial Ecology pipeline (QIIME)" is twice times abbreviated. In the last paragraph of Materials and Methods you can substituted it by "QIIME". The same can be applied by "Flagellin (FliC)", thus it can be replaced with "FliC" the second time that it appears in Materials and Methods. The opposite occurs with the term "OTU" that it does not appear in full in the text. Please, replace "OTU" with "Operational Taxonomic Units (OTU)" the first time that it appears in the text Replace "LPS" with "lipopolysaccharide (LPS)" the first that it appears in the Introduction section and remove other "lipopolysaccharide (LPS)" that can be found thereafter in the text. Replace "Dextran-FITC" with "FITC-Dextran" in the Results section. "kolmogorov-smirnov test" should be in capital letters: "Kolmogorov-Smirnov test"