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ESPS Peer-review Report
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 8132
Title: The role of NADPH oxidase in the pathogenesis of colon inflammation in mice
Reviewer code: 02618027
Science editor: Zhai, Huan-Huan
Date sent for review: 2013-12-19 09:41
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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[ ] Grade A: Priority Publishing	Google Search:	[ ] Accept
[ ] Grade B (Very good)	[Y] Grade B: minor language polishing	[ ] Existed	[ ] High priority for publication
[Y] Grade C (Good)	[ ] Grade C: a great deal of language polishing	[ ] No records	[ ]Rejection
[ ] Grade D (Fair)	[ ] Grade D: rejected	BPG Search:	[ ] Minor revision
[ ] Grade E (Poor)		[ ] Existed	[Y] Major revision
		[ ] No records	

COMMENTS TO AUTHORS

The aim of this study was to explore the role of NADPH oxidase 1 (Nox1) in the oxidative stress-mediated pathogenesis of acute and chronic colon inflammation using a mouse dextran sulphate sodium (DSS) colitis model. The authors nicely show that primary colonic epithelial cells from these animals exhibit: 1) decreased cell viability, 2) increased extracellular hydrogen peroxide release, and 3) increased expression of pro-inflammatory TNF-alpha. Additionally, bacterial byproduct, lipopolysaccharide (LPS), exacerbated colonic epithelial cell derangements associated with acute DSS colitis. The authors purport that treatment with diphenyleneiodonium (DPI) and apocynin demonstrate that Nox1 is critical for colitis-associated colonic epithelial cell derangements. While the data is concise and the manuscript well-written, there are a few comments to be addressed which I feel would enhance the paper and significantly contribute to the field overall: Major comments: 1. The Abstract should have more detailed explanations of the methods, such as describing "BALB/c mice were divided into three groups: 8 mice with acute DSS colitis (3.5% DSS solution, 7 days), 8 mice with chronic DSS colitis (four cycles totaling 44 days of 3.5% DSS solution, 5 days + water, 6 days) and 12 mice without DSS supplementation as control group…" and stating that "…cells were cultivated in the presence of mediators (20 ug/mL of LPS, 1 mM of apocynin, 20 ug/mL LPS + apocynin)…" 2. Representative images of cell viability assays showing viable, apoptotic, and necrotic cells for all experimental groups should be provided. 3. For RT-PCR experiments, the data is presented as 2 x delta Ct which is 2 x (Ct of target - Ct of housekeeping Actb). Based on this calculation, Nox1 mRNA levels in control cells seems to be on par with Actb mRNA levels, even though Nox1 is supposed to be highly expressed in colon cells. What are the endogenous levels of Nox1 in colon cells compared to housekeeping genes? 4. Nox1

produces superoxide, which the authors assert is rapidly converted to hydrogen peroxide. Amplex Red assay measures extracellular hydrogen peroxide release but complementary assays to confirm superoxide production (and therefore, more specifically Nox1 activity), such as ESR, and intracellular hydrogen peroxide assays, such as Amplite Green, would more convincingly show Nox1-mediated oxidant generation. 5. Although Nox1 may be the highly expressed Nox protein in the colon, Nox4 expression and activity has been shown to be increased in the epithelial cells in colon cancer. Since Nox4 is primarily responsible for hydrogen peroxide production, Nox4 expression in both of these acute and chronic DSS colitis models should be evaluated. 6. Neither apocynin nor DPI are specific inhibitors of Nox1. Studies show that apocynin has antioxidant properties and DPI is non-specific flavoenzyme inhibitor. Nox1-null BALB/c mice or Nox1 siRNA experiments would be more definitive in implicating Nox1 in DSS-mediated epithelial colon cell derangements. 7. The authors use DPI in Amplex Red experiments because apocynin interferes with the assay, but does DPI have the same effects as apocynin on cell viability and TNF-alpha? 8. The authors describe more severe clinical symptoms during acute DSS colitis compared to the chronic DSS colitis model, yet Nox1 expression was increased in the cells of the chronic DSS colitis model compared to the acute. Can the authors comment on the relative importance of Nox1 in the consequent clinical symptoms associated with colitis in these models? Are there other mediators of hydrogen peroxide generation that may be involved? Minor comments: 1. There is a typo error in the Discussion section (pg. 12) where "apocynin increased cell viability and decreased TNF-a…" 2. The figure legends of Figs. 2 and 6 state that the mice are 6-8 weeks old, but they are actually older than that in the chronic

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COMMENTS TO AUTHORS

Some major and minor concerns regarding the paper are as follows: Major concerns: 1. Why did the authors decide to use 12 animals for control whereas 8 animals in the treatment group? Also, how many animals per cage were housed? 2. Statistical analysis of histological analysis of colitis is required 3. The authors have mentioned the use of only one endogenous control gene for their RT-PCR experiment. Can the authors confirm if ACTB expression was constant in all samples. 4. The authors, in the introduction part, talk about the effect of pro-inflammatory cytokines on NADPH oxidase expression in intestinal epithelial cells. How does regulatory as well as anti-inflammatory cytokines affect NADPH oxidase? 5. Why did the authors decide to use 12 animals for control whereas 8 animals in the treatment group? Also, how many animals per cage were housed? 6.Statistical analysis of histological analysis of colitis is required 7. The authors have mentioned the use of only one endogenous control gene for their RT-PCR experiment. Can the authors confirm if ACTB expression was constant in all samples. 8. The authors do not specify the experimental unit in their statistical analysis section. This concern is also related to the point no. 2. If each cage was considered as an experimental unit then how many animals were housed per cage? Depending upon the experimental unit, the "n" used for the statistical analysis will change. 9. In table 1, the authors state that the length of colon in control vs. chronic DSS colitis is significant. Please check. Also, it's the ratio of weight over length which has to be shown.. A small description of Bristolscale in materials and methods or result section will be helpful for readers to understand the scale. 10. In the section where the purity of isolated cells is assessed, the authors do not clearly mention details of the markers used. How is it possible to have Tcell marker and monocyte/macrophage marker expression levels same when compared between healthy controls vs.

acute or chronic DSS colitis mice (Figure 2) since colitis is mediated by T cells? This is not explained by the authors anywhere in the text. 11. It is difficult to keep isolated cells in good shape after 3 to 4 h culture. Experiments performed here are beyond this time lapse. The presentation of cell viability data (figure 3) is confusing. One easier way to present this data is setting unstimulated control as 100% and presenting other results relative to the unstimulated control results. The statistical significance presented in the figure is hard to follow. 12. The authors in their cell viability result section state that there is a significant increase in cell viability when LPS is compared to apoc+LPS. This function of Apoc is not discussed later. Does Apoc inhibit cell degeneration in chronic colitis? What mechanism could possibly be involved in this process? Also care should be taken in interpreting the increase in % of viable cells due to Apoc treatment. Unregulated increase in cell viability/ cell proliferation is unwanted as it may indicate cancer. 13. There is no analysis or discussion on possible regeneration of epithelium 14. The presentation of data in figure 4 is very confusing as well as the statistical differences stated in the figure. It is not clear why the authors have evaluated the amount of hydrogen peroxide in cells and biopsies? Please explain. 15. In figure 5, the data presented in each group is highly variable, as indicated by their error bars. Please explain this high variability within group. Unstimulated Chronic DSS colitis vs. LPS stimulated Chronic DSS colitis is significantly not different. 16. The authors have seen no effect on TNF-a production by LPS treated control epithelial cells. Does this indicate that the cells were not stimulated by LPS? How do the authors explain this phenomenon, since LPS is a well-established agent to induce inflammation in

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COMMENTS TO AUTHORS

Comments to the manuscript: The role of NADPH oxidase in the pathogenesis of colon inflammation in mice. The aim of this study, by Rima Ramonaite, et al., was to investigate the role of NADPH oxidase in colon epithelial cells in pathogenesis of acute and chronic colon inflammation using mice dextran sulphate sodium (DSS) colitis model. It is a very important work for application to clinical practice, it was methodologically well developed. Only a few adjustments to the best presentation of the manuscript, results or conclusions are recommended. Abstract: This abstract comprehend 271 words. It is recommended that the authors mentioned in the abstract the name of the NADPH oxidase inhibitors that used in the experiment. Background: In the title the authors present the role of NADPH oxidase in the pathogenesis of colon inflammation in mice. However, in the results, conclusions of the abstract, and also in the introduction they focus the attention in the treatment of NADPH oxidase inhibitors as a protective effect against pro-inflammatory action of lipopolysaccharides (LPS) in colonic epithelium cells of mice with DSS colitis. So it is recommended that the authors to consider a brief modification of the title of the manuscript. Because the title is not only focus is the pathogenesis of NADPH oxidase in the colon inflammation. The reasons are the following: 1) the problem statement (introduction last paragraph) the question of the molecular pathways that control the production of ROS through the products presented in NOX enzymes in the cells of the intestinal epithelium during acute and chronic inflammation. Methods: It is advisable to more clearly describe the methodology definitions of acute and chronic inflammation for the times that were used in the experiment (Second paragraph of methodology, lines 5-7.) In the part of methodology, it is recommended that the authors describe, if the data followed normal distribution and the reasons why the results reported as standard errors and not confidence intervals or standard

deviations. Results: The title of Table 1, is very brief should be completed by noting more information. Writing in Table 1, if the data are presented as mean and SE of the mean. The results of assessment of faeces (points), rectal bleeding and mortality, describe the results in percentages in parentheses. Adequately indicate statistically significant differences with the values of "p". For example what is the value of "p" in the asterisk, and which is the # symbol? It is advisable to write at the bottom of Table 1, the initials that were used. For example, DSS. Complete the subtitle of the second paragraph of results, noting that it is the inflammation of the intestinal epithelial cells. Explain in more detail how the statistical comparisons between groups of acute and chronic inflammation, and the control group were done. It is recommended to the authors write in all the figures, if the data are represented as mean values and standard errors. Discussion The discussion is adequate Conclusions The findings would be largely appropriate if the title is amended as recommended above.