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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 50795

Title: ToxoRO Y6 Ⅰ/Ⅲ induced M2 phenotype of macrophages protects Caco-2 cells via inhibition of M1 associated inflammation

Reviewer's code: 03316915
Position: Peer Reviewer
Academic degree: PhD
Professional title: Professor
Reviewer's country: United States
Author's country: China

Reviewer chosen by: Jin-Zhou Tang
Reviewer accepted review: 2019-08-14 12:49
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Review time: 13 Days

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	[ ] Accept (High priority)	Peer-Review:
[Y] Grade B: Very good	[Y] Grade B: Minor language polishing	[Y] Accept (General priority)	[Y] Anonymous
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Minor revision	[ ] Onymous
[ ] Grade D: Fair	[ ] Grade D: Rejection	[ ] Major revision	Peer-reviewer's expertise on the topic of the manuscript:
[ ] Grade E: Do not publish		[ ] Rejection	[Y] Advanced
			[ ] General
			[ ] No expertise
			Conflicts-of-Interest:
			[ ] Yes
			[Y] No

SPECIFIC COMMENTS TO AUTHORS

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Summary This in vitro study aims to verify the potential role of parasite-derived effector Toxoplasma ROP16 Ⅰ/Ⅲ in ameliorating inflammatory bowel disease (IBD) pathology, through promotes the polarization of M2 macrophages and downregulate the M1 associated inflammatory response. The RAW264.7/Caco-2 co-culture system was established as an inflammatory model of IBD in vitro. the results presented in the manuscript demonstrated that, the RAW264.7 macrophages stimulated by LPS (M1 cells) showed increased production of iNOS, NO, TNF-α, IL-1β, and IL-6 and facilitated Caco-2 cells apoptosis in the co-culture system, while ToxoRop16 Ⅰ/Ⅲ transfected RAW264.7 macrophages bias to M2 cells, enhanced the synthesis of Arg-1, IL-10, TGF-β1, and IL-13. M2 mixed with M1, exhibited downregulation of the pro-inflammatory factors and alleviated Caco-2 cells apoptosis in the co-culture system. According to their experimental results, the authors conclude that the ToxoRop16 Ⅰ/Ⅲ exhibited a protective role on Caco-2 intestinal epithelial cells by promoting the polarization of M2 cells and dampen the M1-mediated inflammatory response. Strength: - The manuscript addressed a promising strategy for IBD immunotherapy with parasite-derived effector ToxoROP16 Ⅰ/Ⅲ. In vitro, the ToxoROP16 Ⅰ/Ⅲ showed a potential to promote M2 cells polarization and ameliorate the M1 mediated inflammatory response. Which is considered a major approach in fighting the IBD. Suggest that the ToxoRop16 Ⅰ/Ⅲ might have potential in ameliorating bowel inflammation by driving intestinal epithelial macrophages to M2 cells phenotype and maintaining the equilibrium of the gut macrophage subsets. - The experiments are well designed in term of sampling, control and data validation. Weakness: - To verify the role of ToxoRop16 Ⅰ/Ⅲ on intestinal homeostasis, the authors only examined the Caco-2 cells apoptosis, which not necessarily reflect the functionality of the Caco-2 cells. It would be worth to examine the effect of ToxoRop16 Ⅰ/Ⅲ on Caco-2 monolayer integrity and permeability. - The authors used a co-culture system of the intestinal epithelial Caco-2 cells (human cell line)

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and macrophage RAW264.7 cells (mice cell line), which raise a concern about the applicability of this model. Besides, the authors didn't mention the origin of the cell lines used in the manuscript. - In the co-culture system, the RAW264.7 cells were seeded at a density of 2×106 cells. Whereas, the Caco-2 cells were seeded at a density of 5×105 cells, 4:1 ratio, which could increase the Caco-2 burden in this model. - The manuscript included an insufficient description of the Caco-2 differentiation status which could remarkably affect its response. - The Arg-1 relative mRNA and protein expression results are not consistent. In figure (5, C) the relative mRNA expression of Arg-1 was markedly higher in LV-rop16 Ⅰ/Ⅲ-Mφ group (8-fold) compared with M1+M2 group. Whereas, in figure (7.C) the Arg-1 protein expression was higher (2-fold) in M1+M2 group compared with LV-rop16 Ⅰ/Ⅲ-Mφ group. Furthermore, in figure (4, G) the Arg-1 protein expression was 2-fold higher in LV-rop16 Ⅰ/Ⅲ-Mφ group relative to Mφ, compared with 1-fold higher expression in figure (7.C). - The authors should correct the figures numbering, for example, the protein expression of Arg-1 and PD-L2 were presented in figure 7 not figure 6 as it was mentioned in the manuscript. - In the methods; the NO assay section, the sampling and the density of the cells were ambiguous and unclear.

INITIAL REVIEW OF THE MANUSCRIPT

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Reviewer's code: 02529364
Position: Editorial Board
Academic degree: MD, PhD
Professional title: Senior Lecturer
Reviewer's country: Australia
Author's country: China
Reviewer chosen by: Jin-Zhou Tang
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Reviewer performed review: 2019-09-05 00:26
Review time: 20 Days and 12 Hours

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection	Peer-Review: [Y] Anonymous [ ] Onymous Peer-reviewer's expertise on the topic of the manuscript: [ ] Advanced [Y] General [ ] No expertise Conflicts-of-Interest: [ ] Yes [Y] No

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In this manuscript, the authors verified previously reported finding by other researchers that ToxoROP16 I/III induced M2 polarization in RAW264.7 macrophage cell line. They further showed that ToxoROP16 I/III induced M2 phenotype of RAW264.7 cells reduced the apoptosis in intestinal epithelial cell line Caco2 cells induced by M1 phenotype of RAW 264.7, the M1 type RAW264.7 cells were pre-induced using LPS. The data reported in this manuscript were generated using a cell culture model, which are difficult to directly related to IBD. However, the observation that a component of Toxoplasma gondii polarized M2, which in turn inhibited M1 induced epithelial damage is still interesting. Please see my comments below: 1. The manuscript has no page numbers and no line numbers, which makes it very difficult to communicate between the reviewers and the authors. 2. Conclusion in the abstract: "These findings may be helpful for gaining a better understanding of the underlying mechanism". What mechanism do the authors refer to? 3. Introduction: "with changes to living conditions, the incidence of IBD is increasing". Do the authors mean the incidence of IBD in China? 4. "During the process of inflammation, macrophages play a central part in cell polarization". Please specify which cell polarization. 5. "Jensen previously demonstrated (28)". The first author of reference 28 is Melo MB, why was the second author mentioned? 6. Table 1: references should be included about who have designed these primers. 7. Figure 5. Why TNFa was not there? 8. Discussion: ROP16 is a kinase that can directly phosphorylates the Stat3/Stat6. Does ToxoROP16 I/III contain the full gene encoding ROP16 or only a fragment?

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