Dear Editor,

Thank you for considering the manuscript entitled "Research advances of vasoactive intestinal peptide in the pathogenesis of ulcerative colitis by regulating interleukin-10 expression in Bregs" (59785). We really appreciate all the valuable comments and constructive suggestions from reviewers. We have revised the manuscript and a point-by-point response was enclosed. Revised portion are marked in blue in the paper. We would like to re-submit the revised manuscript to the World Journal of Gastroenterology, and hope it is acceptable for publication in the journal. Please do not hesitate to contact us for any question or concern.

We look forward to your final decision.

Sincerely yours

Yan-Cheng Dai

Responses to reviewer:

Reviewer #1: This is a well written review paper concerning the role of vasoactive intestinal peptide (VIP) in regulatory activity on intestinal immune response and its possible role in pathophysiology of ulcerative colitis. The authors have reviewed and analyzed a sufficient amount of literature (55 articles). In this review authors described well the role of regulatory B cells (Bregs) and IL-10 in intestinal immunity in ulcerative colitis patients, and pay attention on contribution of B cells to Treg homeostasis and their interactions in prevention of excessive immune response by colitis. In this review is well described the role of IL-10 in maintaining intestinal homeostasis and precisely described the role of VIP in regulation of expression of IL-10 in Bregs. Also the authors underlines that it should be taken into consideration the fact that there are yet many open pathophysiological mechanisms in behavior and co-stimulatory mechanisms concerning regulation of IL-10 expression by VIP in Bregs in pathogenesis of ulcerative colitis. Concerning the quality of this

review paper it would be necessary also to mention in some words the methods which might estimate the detection of VIP, Bregs and Il-10 in ulcerative colitis patients.

Response: We have added the methods which might estimate the detection of VIP, Bregs and Il-10 in ulcerative colitis patients in the conclusion part of the manuscript. Thank you for your valuable advice.

Reviewer #2: In the review manuscript the authors aimed to summarize the current literature on the pathogenic role of VIP and Bregs in UC. The topic is of clinical importance, however, the presentation of the review need major revision. My comments to the authors: -The term „nodal-like" must be corrected to NOD-like -„Adaptive immunity is a highly specific and adaptive type of immunity." This sentence must be rephrased to be more scientific - In the Intorduction the authors must pointed out why they review the role of VIP and Bregs in the context of UC pathogenesis. -"IL-10 is expressed in different cells involved in innate and adaptive immunity" – What are these cells? - In case of VIP the author describe that „VIP treatment reduced the levels of various chemokines and pro-inflammatory cytokines"- What are they? It is important to understand their pathogenic role. - The authors wrote: „Both T and B lymphocytes express the VIP-related receptors[41], suggesting that B lymphocytes may regulate their immune response through VIP[42]. „ This must be modified like this: „Both T and B lymphocytes express the VIP-related receptors[41], suggesting that B lymphocytes may regulate their immune response partly through VIP[42]." - What does „non neurons" means? - „In innate immunity, VIP inhibits the production of inflammatory cytokines and chemokines by immune cells, reduces the expression of co-stimulatory molecules on antigen-presenting cells" – what are these cytokines, chemokines an co-stimulatory molecules? - The use of TNBS-colitis in the

review is confusing: sometimes it is referred as an animal model of Crohn's disease, sometimes it is mentioned regarding to UC. This must be clarified. -A „Future perspective" is missing form the review: the systhesis of the VIP-Breg axis to the pathogenesis and possible therapeutic options of UC should have incorporated to strengthen the manuscript. - The title of the manuscript must be changed since the current title „Recent advances in research of vasoactive intestinal peptide and ulcerative colitis" in contrast of the last sentence of Conclusion „With further clarity on the mechanism of the regulation of IL-10 expression by VIP in Bregs of colitis patients, we believe that Bregs would provide a novel strategy for the clinical treatment of UC." does not reflect the importance of the role of Bregs. - English language needs minor polishing. After major revision I suggest to accept the manuscript for publication in WJG.

Response: We have revised this mistake in the manuscript. We have revised the title of the article to " Research advances of vasoactive intestinal peptide in the pathogenesis of ulcerative colitis by regulating interleukin-10 expression in Bregs ". We have revised the last sentence of conclusion according to reviewer's suggestion." With further clarity on the mechanism of the regulation of IL-10 expression by VIP-Breg axis of colitis patients, we believe that VIP would provide a novel strategy for the clinical treatment of UC."

Thank you for your valuable advice.

Reviewer #3: Your well-written REVIEW of Recent Advances of VIP in CUC is an expansion of current knowledge of the pathophysiology of CUC (TH1/TH2 balance) by Bregs [regulatory B cells] which negatively regulate intestinal immunity. You go on to suggest that VIP regulates IL-10 expression in colitis patients and may provide a novel strategy to treat CUC. Current PubMed review lists 5 articles or abstracts on VIP in the treatment of CUC between 1993 – 2014 and 12 abstracts and publications looking at the role of VIP in IBD. I was able to find a single publication by Wang et al reporting a

decrease in regulatory B cells in CUC (J Crohns Colitis 2016;10:1212–23) and another that VIP stabilizes intestinal homeostasis through maintaining IL-10 expression in regulatory cells (Sun et al. Theranostics 2019;9:2800-2811) [reference 54]. Despite the fact that a number of your references had to do with RA, asthma, or autoimmunity in general, your manuscript builds a credible role for Bregs, VIP, and IL-10 in bowel homeostasis, and the potential role of VIP stabilized micelles (VIP-SSM) for CUC therapy in the future.

Response: Thank you for your comments.

Reviewer #4: The review addresses the role of vasoactive intestinal peptide in the pathogenesis of ulcerative colitis. An overview about VIP is given and its regulatory aspects are addressed. Comments 1. It comes not clear to the reader, why VIP is probably regulative in ulcerative colitis but not in the different types of Crohn's disease. This point should be addressed. 2. The scheme/ graphical abstract is very simple and should be improved.

Response:

Comments 1.

T Helper 2 cell (Th2) is a T cell subpopulation that secretes Th2 type cytokines (such as interleukin-4, IL-5, IL-10, and IL-13, etc.). These cytokines can promote Th2 cell proliferation, inhibit Th1 cell proliferation, assist B cell activation, and play the role of humoral immunity.

Studies have found that $CD4^+$ T cells in inflammatory intestinal mucosa of patients with CD produce a large number of Th1 effect proinflammatory cytokines (such as IFN-γ, TNF-α, IL-2) after stimulation in vitro, while $CD4^+$ T and NK-T cells in inflammatory intestinal mucosa of UC patients can secrete a large number of Th2 effector cytokines (such as IL-4, IL-13). It is currently accepted that CD is primarily associated with Th1-mediated responses, while UC is primarily associated with Th2-mediated responses (Bin Xia, Chang-Sheng Deng, Kai-Chun Wu, Bo Shen. Inflammatory bowel disease

(The third edition) [M]. Beijing: People's Medical Publishing House. 2015: 117.Chinese).

Regulatory B cells secretion of IL-10 can induce Th0 cells to develop into Th2 cells,Inhibiting Th1 cytokines development, thereby affecting Th1/Th2 balance, we research on human peripheral blood specimens and animal experiments found that in the intestinal microenvironment of UC,VIP deficiency can accelerate the degradation of IL-10 mRNA level, resulting in Breg dysfunction.VIP plays an important role in stabilizing IL-10 mRNA expression in Bregs, and giving VIP can effectively inhibit experimental colitis in mice(Reference 54). Therefore, we believe that VIP may regulate ulcerative colitis, but it does not play a major role in Crohn's disease.

Comments 2.

The abstract part of the manuscript has been revised. Thank you for your valuable advice.