

Point-by-point response to each of the issues raised in the peer review reports:

Manuscript:

"Recent insights into the characteristics and role of peritoneal macrophages from ascites of cirrhotic patients" NO.: 67093, Frontier (WJG).

Reviewer: 1

Comments to the Author

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: This was a review article for the section FRONTIER of the WJG. There are 3 figures, no tables, 52 references. The Authors have previously investigated several pathways of peritoneal macrophages; therefore they actually depict a concise, well-written overview on this topic. Moreover, as stated in the Title, they tried to offer new insights on this translational research, citing recently published articles. In my opinion, the paper is not redundant and falls into the scope of the Frontier section of the WJG.

Author's answer:

We thank the reviewer for taking the time to review our manuscript, and really appreciate the positive and constructive considerations and comments to improve its quality. These suggestions have been incorporated in the revised version of the manuscript.

Comments to Authors: - I would not report the section about omentum.

Author's answer:

Following Reviewer's suggestion the section on omentum has been withdrawn from the revised version of the manuscript.

Comments to Authors: I agree with the fact that CAPD patients are not healthy people, and that collection of peritoneal fluid is not possible in healthy people. How the Authors explain the peritoneal fluid they analyzed during gynaecological interventions? Can we assume that this peritoneal fluid can be due to a certain degree of inflammation?

Author's answer:

Under healthy conditions, a small volume of 5–20 mL peritoneal fluid is physiologically present in the peritoneal cavity. This is a mixture of plasma transudate and ovarian exudate.

Furthermore, tubal fluid and macrophages' secretions contribute to the peritoneal fluid (Oral et al., 1996; Van Baal et al., 2017; Heel KA et al., 2017). This liquid is responsible for the physiological function of lubricating the friction of the intestinal loops and other organs contained in the peritoneal cavity. Furthermore, our healthy peritoneal cell samples were mainly obtained during laparoscopies for tubal ligation, although we also included some patients undergoing therapeutic laparoscopies or laparotomies for benign gynecological pathologies, such as simple ovarian cysts. In our experience, the first peritoneal fluid obtained by the endoscopic aspirator is quite scarce; with a mean of 6.85 ± 2.6 mL (range 5–8.7 mL). Moreover, this fluid practically lacks PMNs, which is indicative of the absence of local inflammatory signals that would have induced the migration of these cells into the peritoneal cavity (See Fig. 2 in Ruiz-Alcaraz et al., 2020. doi: 10.1111/imcb.12305).

Comments to Authors: - Clinical diagnosis of spontaneous bacterial peritonitis is based on quantitative measurement of PMN cells of ascites. Any information about the quantitative role of ascitic fluid macrophages?

Author's answer:

Under normal conditions, peritoneal macrophages comprise 50-90% of the peritoneal leucocytes and are primarily responsible for clearing debris and pathogens. As it is indicated now in the revised version of the manuscript, among human peritoneal leukocytes, macrophages are the predominant cell type (45–90%) followed by T lymphocytes (predominantly T effector/memory cells, CD45RO) (45%), NK cells (8%), Dendritic cells (2–6%), B lymphocytes (2%) and less than 5% of PMN cells (See Ruiz-Alcaraz et al., 2020 and Kubicka et al., Scand J Immunol 1996;44(2):157–63).

Also, according to Nieto et al., 2015, "the ascites from the NegC-SBP and PosC-SBP patients had significantly more macrophages with low SSC than those from SA patients (percentage of PosC-SBP, $79.70 \pm 6.07\%$; percentage of NegCSBP, $79.81 \pm 4.84\%$, and percentage of SA, $58.10 \pm 6.87\%$; PosCSBP vs. SA, $P = 0.03$; NegC-SBP vs. SA, $P = 0.02$; Fig. 3B). Macrophages from PosC-SBP and NegC-SBP ascites, predominantly with CD14^{low} expression, had an impaired phagocytosis (MFI 26.67 ± 10.75 and 31.81 ± 4.26 , respectively). A direct consequence of this phenotype is the poor bactericidal capacity of PosC-SBP macrophages and the tolerant state of the macrophages." (See also Runyon, B. A. and Van Epps, D. E., 1986. Hepatology 6,396–399.27 and Such, J. et al., 1988. J. Hepatol.6,80–84).

Finally, according to Fagan et al., 2015: "High ascites bacterial burden is associated with reduced macrophage HLA-DR expression. The presence of monocytes/macrophages (CD14⁺/HLA-DR⁺) in ascites fluid was associated with lower ascites neutrophil numbers and a trend towards lower bacterial burden ($r_s = -0.59$, $p = 0.011$ and $r_s = -0.41$, $p = 0.081$ Fig. 5B and data not shown). Consistent with the hypothesis that innate immune function is impaired in

patients with cirrhosis, HLA-DR expression on ascites CD14^{Hi}/HLA-DR⁺ monocytes/macrophages inversely correlated with bacterial DNA levels ($r_s = -0.48$, $p = 0.04$, Fig. 5C)".

Comments to Authors: - Does any comparison exist between macrophages in cirrhosis with ascites and macrophages in cirrhosis with ascites and SBP? Any association between macrophages and degree of liver function (e.g., according to Child-Pugh score)?

Author's answer:

As far as we know there is not a direct comparison between macrophages in cirrhosis with ascites and macrophages in cirrhosis with ascites and SBP. We did not analyse this point because SBP was, in fact, one of our exclusion criteria. Nevertheless, we analysed two samples from SBP prior to the confirmation of the clinical stage of those patients. In these two cases the total number of leukocytes was higher and the macrophage phenotype tended to be more proinflammatory. These data agree with the results of Nieto et al., 2015.

Regarding association between macrophages and degree of liver function (e.g., according to Child-Pugh score) we do not have data on this point. Nevertheless, our published data from 45 patients with cirrhosis and culture-negative ascites showed that the number of macrophages in ascites were different between alcohol and hepatitis C aetiology, although the Child-Pugh mean score was similar in these two groups of patients with different aetiology (See Tapia-Abellan et al., 2012. DOI: 10.1186/1471-2172-13-42).

Comments to Authors: - I agree with the hypothesis that intermittent bacterial translocations may induce a "preactivated" status of macrophages. An underlying, persistently activated - although subclinical - inflammatory status has been proposed as a driver for decompensated cirrhosis (as explained in this paper: doi 10.1016 / j.jhep.2020.11.048). A comment on this topic would be valuable.

Author's answer: Following Reviewer's comments, we have added a further comment about this topic in the Introduction of the revised version of the manuscript, acknowledging the contribution of the mentioned paper (page 5).

Comments to Authors:

Minor:

- there are few typos (e.g., Fig3, presets)

Author's answer: Typographical errors have been corrected.

- Page 17: Saying that "sCD206 is an independent predictor of death in patients with SBP" may be more appropriate.

Author's answer: This paragraph has been modified as suggested by the reviewer.

- abbreviations are not cited in the abstract body.

Author's answer: Abbreviations have been revised and cited in the whole manuscript.

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: The authors summarized recent relevant publications on the biology of peritoneal macrophages under the perspective of its future clinical translation to the role that these cells can play on several human liver diseases. The manuscript is interesting and suggested to be published in this journal.

Author's answer:

We thank the reviewer for taking the time to review our manuscript, and really appreciate his/her positive considerations about it.