Point by point response

Dear editor and reviewers,

We are very thankful for your constructive comments.

Indeed, using them we have tried to ameliorate this current version of the manuscript. We would like to draw your attention to the fact that, unlike mesenchymal stem cells (MSCs), our knowledge of the immunoregulatory function of the endothelial progenitor cells (EPCs) is very limited and our research unit was among the first to investigate and report this biological feature. Therefore, the paucity of the information on this part is due to the absence of the previous background.

Working on both EPCs and MSCs in recent years we have been able to directly compare them regarding their immunosuppressive and immunoregulatory functions against T cells.

Please find below our responses to your raised questions.

Regards

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Reviewer #1:
Scientific Quality: Grade C (Good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Major revision

Specific Comments to Authors: In this review, the authors propose a comparison between bone-marrow-derived mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs) related to immunoregulatory effect of these two populations. The work is well organized in its parts. Overall, this work is interesting, underlining the key point of MSC and EPC immunoregulatory and it could provide a starting point for further studies aimed at the development of innovative drugs and therapies.

- We are thankful for this summary that truly reflects our messages.

However, there are some points to address:

The authors reported substantially only their own data about EPC immunomodulation. Are they sure that further data are not present in the literature? Otherwise they should be included in the review.

We are thankful for this comment which is indeed correct. As explained at the beginning of this letter, the existing information on the immunological aspects of EPCs is extremely limited. Most of the publications using these cells in an *in vivo* condition are using immunodeficient mice to avoid immunological reactions. We have shown for the first time that when these cells from human sources are administered into **immunocompetent mice**, they can be found even 30 days after at the site of endothelial tissue damage. This shows that even in a highly inflammatory xenogenic environment they can home and exert their proper function. Further investigations in our team revealed that they are indeed able to secrete IL-10, TGFβ, and HLA-G which are known anti-inflammatory mediators that enable them to suppress xenogenic and also allogeneic (human-human) PBMCs and especially T cells.

As suggested, we have added a limited number of other works that demonstrate EPC immunoregulatory function.

We have now added this work to page 4 lines 14 to 28.

" Unlike MSCs, there are not many studies to evaluate the immunogenicity and immunoregulatory features of EPCs and their interaction with the immune system. Most of the previous studies have used EPCs to restore blood perfusion notably in hind limb ischemia condition that was performed in immunodeficient mouse models to avoid potential immunological responses.[53–55] Eugenia R Nussolo et al, have already demonstrated that EPC derived from CB has a significantly lower pro-inflammatory and pro-thrombotic profile than adult EPCs.[56] Some limitations of this work are that EPCs were not compared to mature ECs, thus, one cannot observe whether the reported results are progenitor dependent or not. Furthermore, these evaluations were at the gene expression level which can be different compared to the protein level. In an allogenic combination, Juliane Ladhoff et al, have demonstrated that rat EPCs are immunotolerated against allogeneic immune responses and particularly humoral-mediated attacks *in-vitro*. Furthermore, when they transplanted these cells as a component of a vascular graft, allogenic EPCs were not rejected.[57] However, the interaction of the immune system, notably, T cells with EPCs remains unclear. In an attempt to clarify these missing

pieces of information, we have recently reported that EPCs can also regulate the immune response and bear some level of immunoregulation, especially against T cells.[40] ”

In 2.3 section the authors should introduce, also in minimal part, TNFα and TNFR2 related to general aspect.

Further explanation about TNFR1 and TNFR2 was added to the 2.3 section as requested.

Please refer to page 7 lines 26 to 34.

”TNFα recognizes two transmembrane receptors (TNFR1 and TNFR2) with two completely distinct biological functions.[100–102] While the interaction of TNFα with TNFR1 leads to pro-apoptotic and deleterious outcomes, its interaction with TNFR2 generally causes cell activation, proliferation, and survival.[101–103] TNFR1 and TNFR2 are different subgroups of the TNF receptor superfamily (TNFRSF).[104] TNFR1 is a death receptor since it bears a death domain (DD) in its cytoplasmic compartment and its activation ends in caspase-8 function and cell death.[105–107] TNFR2 on the other hand, recruit TRAF2 with its associated binding molecules such as cIAP1, cIAP2, and TRAF1 which results in the activation of the classical NF-kappa B (NFκB) and mitogen-activated protein kinases (MAPK) pathways leading to cell proliferation.[106,108]”

Also, in the conclusion part of this review the authors should highlight mostly therapeutic implications about TNF–TNF Receptor 2.

This point is now added as requested.

‘’ Due to its protective and anti-inflammatory role, activation of the TNFR2 axis has been suggested as a therapeutic approach in several degenerative, inflammatory and cardiovascular disorders. The stimulation through the TNFR2 molecule has been shown as a promising approach to increase the pro-angiogenic effect of TNFR2 expressing cells leading to improved ischemia conditions,[113,114] myocardial infarction,[115,116] Alzheimer's disease.[117] Similar outcomes were reported regarding improved Treg immunosuppressive function which could potentially improve GVHD,[118] or autoimmune disorders.[110] Conversely, the TNFα-TNFR2 axis was used as a potential target for Treg elimination in cancer conditions in which elevated immune response is required.[111,119,120] This is indeed very interesting since the Foxp3 molecule is a transcription factor (i.e. intranuclear) and not easily accessible for Treg elimination, therefore, targeting TNFR2 (with cytoplasmic expression) seems to be an efficient alternative to hamper immunosuppression in Tregs and also in other immunomodulatory cells such as EPCs and MSCs. ”

Table 1 and Table 2 are not very explanatory, the authors should explain the criteria used to create the tables and the value assigned.

Thanks for raising this important question.

Here in this review article, we have decided to compare the immunomodulatory effect of MSCs and EPCs from fetal and adult sources. Our research unit has been studying the immunological properties of these cell types for years in which we have published several original articles. Therefore, we have decided to base the entire evaluation on our internal experimentations which makes it possible to directly compare their immunoregulatory effect.

Concerning table 1, according to our experience, among the four cell types (FL-MSCs, BM-MSCs, CB-EPCs, and APB-EPCs) FL-MSCs are the most immunomodulatory cells since we observed a more 1) suppression of the T cells’ proliferation, 2) decrease in T cells’ activation

phenotype and 3) decrease in T cells' secretion of pro-inflammatory cytokines. On the other hand, they could more efficiently increase 1) the secretion of anti-inflammatory cytokine and 2) the induction of Tregs, than the other cells (Yi Yu et al 2021, Naserian et al 2020, Naserian et al 2019, Nouri-Barkestani et al 2021, Beldi et al 2020, Beldi et 2020). Therefore, we have considered them as the reference (5 points/5 points) regarding the measured criteria and compared the capacity of the other cells with FL-MSCs. In the case of CB-EPCs and APB-EPCs, since we did not notice any Treg induction or elevation of anti-inflammatory cytokine secretion we attributed a 0/5 score.

Concerning the second table, since the immunosuppressive activity of MSCs is greater than EPCs, we have kept them as the reference (5/5). In this case, in the presence of the TNFα-TNFR2 signaling pathway, normal condition, MSCs have the highest immunosuppressive effect (5/5). On the other hand, in the presence of this signaling, EPCs have a less immunosuppressive effect (3/5). Interestingly, while blockade of this axis led to a complete loss of immunosuppressive function in EPC (0/5), MSCs kept suppressing T cells with less efficiency (2/5), showing that this axis is partially controlling their immunoregulatory properties. (Naserian et al 2020, Nouri-Barkestani et al 2021, Beldi et al 2020, Beldi et 2020)

A complementary explanation is now added to table legends summarising the taken criteria.

The authors should expand 2.2 section introducing Treg cells and pathway related to Treg induction.

We are thankful for this comment. As requested we have now expanded this section. Please refer to page 7, the new section 2.2. ''MSCs and EPCs have different Treg induction capacity.''

Reviewer #2:
Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Minor revision

Specific Comments to Authors: It is a nicely written review on an interesting topic, the comparison between MSCs and EPCs on their immunoregulatory abilities could be useful in guiding future application.

We are very thankful for this reviewer's grades and comments.

Some questions are raised as follows: Page 3, line 42: Some abbreviations were explained at their first appearance while some were not, for instance, CB. I understand that there is a list of abbreviations, but it is better to unify the format.

We are very sorry for these unseen mistakes. We have now verified all the abbreviations and explained them for their first time.

Page 4, line 12: It is better to add a citation of the website.

Thanks for this suggestion. As requested we have first updated and then added a hyperlink to direct readers to the website. Please visit the following link to observe the clinical trials based on EPCs.

https://www.clinicaltrials.gov/ct2/results?recrs=&cond=&term=endothelial+progenitor+cells&cntry=&state=&city=&dist=

Table 1 and 2: How were the abilities quantified?

Thanks for raising this important question.

Here in this review article, we have decided to compare the immunomodulatory effect of MSCs and EPCs from fetal and adult sources. Our research unit has been studying the immunological properties of these cell types for years in which we have published several original articles. Therefore, we have decided to base the entire evaluation on our internal experimentations which makes it possible to directly compare their immunoregulatory effect.

Concerning table 1, according to our experience, among the four cell types (FL-MSCs, BM-MSCs, CB-EPCs, and APB-EPCs) FL-MSCs are the most immunomodulatory cells since we observed a more 1) suppression of the T cells' proliferation, 2) decrease in T cells' activation phenotype and 3) decrease in T cells' secretion of pro-inflammatory cytokines. On the other hand, they could more efficiently increase 1) the secretion of anti-inflammatory cytokine and 2) the induction of Tregs, than the other cells (Yi Yu et al 2021, Naserian et al 2020, Naserian et al 2019, Nouri-Barkestani et al 2021, Beldi et al 2020, Beldi et 2020). Therefore, we have considered them as the reference (5 points/5 points) regarding the measured criteria and compared the capacity of the other cells with FL-MSCs. In the case of CB-EPCs and APB-EPCs, since we did not notice any Treg induction or elevation of anti-inflammatory cytokine secretion we attributed a 0/5 score.

Concerning the second table, since the immunosuppressive activity of MSCs is greater than EPCs, we have kept them as the reference (5/5). In this case, in the presence of the TNFα-TNFR2 signaling pathway, normal condition, MSCs have the highest immunosuppressive effect (5/5). On the other hand, in the presence of this signaling, EPCs have a less immunosuppressive effect (3/5). Interestingly, while blockade of this axis led to a complete loss of immunosuppressive function in EPC (0/5), MSCs kept suppressing T cells with less efficiency (2/5), showing that this axis is partially controlling their immunoregulatory properties. (Naserian et al 2020, Nouri-Barkestani et al 2021, Beldi et al 2020, Beldi et 2020)

A complementary explanation is now added to table legends summarising the taken criteria.

Further language editing is required.

As requested, we have now performed English language polishing after performing the amendments.

The manuscript appears to be short for a review.

We are thankful for this comment. Indeed, we are aware that this manuscript is rather short. As explained earlier, the paucity of information on EPC immunological properties limited us to go further into details. On the other hand, many previous review articles already exist about MSC immunological function. Thus, we would like to avoid repeating the MSC-related subjects.

We have now added some complementary and more detailed information throughout the manuscript as requested by the reviewers.

Reviewer #3:
Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Accept (General priority)

Specific Comments to Authors: The author of this paper has rich experience in the study of EPCs and MSCs, and confirmed that they have a certain level of immune regulation function. For the first time, there are similarities and differences between them in T cell immune regulation. It is confirmed that MSCs have stronger immunosuppressive and immunosuppressive effects, and immunomodulators are better than CB and APB EPCs. And the TNFα-TNFR2 axis leads to the complete disruption of EPC and has a significant impact on MSC immunomodulatory functions. To provide a theoretical basis for clinical application in cancer, transplantation and inflammatory diseases in the future. This is indeed a very interesting study, on the basis of which we can further study the immune interaction between MSCs and EPCs and T cells, and we believe that there will be more harvest.

- We are very grateful for this reviewer's opinions.

Science editor: 1 Scientific quality: The manuscript describes a minireview of the differences and similarities between MSC and EPC immunoregulatory properties against T cells. The topic is within the scope of the WJSC. (1) Classification: Two Grades B and Grade C; (2) Summary of the Peer-Review Report: It is a nicely written review on an interesting topic, the comparison between MSCs and EPCs on their immunoregulatory abilities could be useful in guiding future application. The questions raised by the reviewers should be answered; (3) Format: There are 2 tables and 1 figure; (4) References: A total of 73 references are cited, including 25 references published in the last 3 years; (5) Self-cited references: There are 18 self-cited references. The self-referencing rates should be less than 10%. Please keep the reasonable self-citations (i.e. those that are most closely related to the topic of the manuscript) and remove all other improper self-citations. If the authors fail to address the critical issue of self-citation, the editing process of this manuscript will be terminated; and (6) References recommendations: The authors have the right to refuse to cite improper references recommended by the peer reviewer(s), especially references published by the peer reviewer(s) him/herself (themselves). If the authors find the peer reviewer(s) request for the authors to cite improper references published by him/herself (themselves), please send the peer reviewer's ID number to editorialoffice@wjgnet.com. The Editorial Office will close and remove the peer reviewer from the F6Publishing system immediately. 2 Language evaluation: Classification: Three Grades B. 3 Academic norms and rules: No academic misconduct was found in the Bing search. 4 Supplementary comments: This is an invited manuscript. The study was supported by Agence Nationale de la Recherche, la Fondation de la Maison de la Chimie. The topic has not previously been published in the WJSC.

5 Issues raised: (1) The "Author Contributions" section is missing. Please provide the author contributions; (2) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s); (3) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor; (4) PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout; and (5) If an author of a submission is re-using a figure or figures published elsewhere, or that is copyrighted, the author must provide documentation that the previous publisher or copyright holder has given permission for the figure to be re-published; and correctly indicating the reference source and copyrights. For example, "Figure 1 Histopathological examination by hematoxylin-eosin staining (200 ×). A: Control group; B: Model group; C: Pioglitazone hydrochloride group; D: Chinese herbal medicine group. Citation: Yang JM, Sun Y, Wang M, Zhang XL, Zhang SJ, Gao YS, Chen L, Wu MY, Zhou L, Zhou YM, Wang Y, Zheng FJ, Li YH. Regulatory effect of a Chinese herbal medicine formula on non-alcoholic fatty liver disease. World J Gastroenterol 2019; 25(34): 5105-5119. Copyright ©The Author(s) 2019. Published by Baishideng Publishing Group Inc[6]". And please cite the reference source in the references list. If the author fails to properly cite the published or copyrighted picture(s) or table(s) as described above, he/she will be subject to

withdrawal of the article from BPG publications and may even be held liable. 6 Recommendation: Conditional acceptance.

Dear Editor,

Thanks for these comments. We have now changed the requested issues accordingly.

- Concerning the self-citations, since most of the evaluation and data used in this manuscript is based on our internal experimentation and especially for those of EPCs no further publication exists, we are obliged to use them to build up our message. We have now kept the 12 most important citations that respect the 10% of the self-citation policy.
- The authors' contribution section was already provided in the manuscript. Please refer to page 12 lines 17 to 19:

''Authors' contributions

S.N conceived the subject and determined the different sections of the manuscript. M.R, M.KH, SH.B, S.SH, and S.N wrote the manuscript. G.U, S.SH, and S.N revised the manuscript.

All authors read and approved the final manuscript.''

- We have now provided the approved grant application form.
- We have now provided original files in ppt format as requested.
- We have now used the citation style of the **World Journal of Stem Cells (WJSC)** for the Zotero application that represents PMID and DOI if available for each publication.
- This manuscript does not use any images or figures from any other publications.