Ministério da Saúde

FIOCRUZ
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Centro de Pesquisa Aggeu Magalhães

Dr.

Editor-in-Chief

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Dear Lian-Sheng Ma,

Thank you for your email dated on 02/09/2017 regarding our manuscript # WJG- 32302. We have revised the manuscript according to the referee's suggestions. Please find below a point by point of answers to the referee's comments. We believe the manuscript is greatly improved and hope you will find it acceptable now for publication at WJG.

Sincerely yours,

Sheilla Andrade de Oliveira

Sheilla Andrade de Oliveira, Ph.D

Titular Research in Public Health
Aggeu Magalhães Institute
Oswaldo Cruz Foundation- FIOCRUZ

Referee's comments (2613702)	**Answers**
Please provide histological sections of normal mice (i.e., not treated with CCl4/ethanol) and the corresponding subsequent analyses for the experiments presented in Fig. 2, 3, and 4 (as a comparative control esp. for the monocyte-treated hepatic injury model mice). -	Histological sections and analyses for the experiments presented in Fig. 2, 3, and 4 of normal mice were added according to referee's suggestion
To further assess the impact of GSH amounts on oxidative stress in the different groups, the levels of ROS in the livers of the respective mice have to be determined (e.g., by dihydroethidium staining)	Dihydroethidium staining followed by confocal microscopy analysis was carried out in frozen tissues according to the reviewer suggestion. However, this technique was rather unspecific staining the whole cell instead of mitochondria only as it is supposed to stain. This lack of specificity was observed even when low concentrations of dihydroethidium were used. Unfortunately, direct measurement of ROS in tissues using fluorescent probes has several issues regarding lack of specificity. Despite being an indirect measure of oxidative stress, GSH measurement is quite reliable, and has been widely accepted as a good indicator of changes in ROS levels.
The text of the manuscript has to be revised considerably (typos, missing/redundant spaces, introduction of abbreviations, wording and style, …).	The manuscript was revised according to referee's suggestion
Please revise Figure 5C. According to the Figure Legend, levels of IL-6 in the liver are shown, but the bar diagram appears to be identical to Figure 5B (showing levels of IL-1β).	We apologize for the mistake and it is now corrected

Referee's comments (1852132)	**Answers**
To demonstrate the biological significance of monocytes in the treatment of liver fibrosis, mice suffering from liver fibrosis were transfused with 106 BMMC or with BMMC-derived from CD11bhi monocytes.	Excellent suggestion. In our study (data not shown) we used a control group that received as therapy the cells that were not retained in the magnetic microbeads. The analysis of this experiment showed results similar to those

Statistically significant differences in fibrosis could be detected in group 1 and 3, but the changes in cytokine levels was detected exclusively in case of IL-13. This result raises the possibility that the limited effect of BMMC on hepatic fibrosis (group 2) may be due to the lower ratio of monocytes as compared to the enriched monocyte population (group 3). Thus, to determine the real impact of monocytes in the treatment of liver fibrosis, further control experiments and additional treatment samples should be involved in the study. For example, it would important to determine the baseline levels of the analyzed parameters in healthy mice followed by measuring the effects of monocyte-depleted BMMC transfusion in mice with chronic liver fibrosis. The results of such control experiments could significantly increase the impact and significance of this study.	obtained with mononuclear cells. We decided to perform the cellular characterization of this population and found that 38.93% of the cells were $CD11b^{+}CD14^{+}$ monocytes. In this way we could not evaluate the therapy without the presence of $CD11b^{+}CD14^{+}$ monocytes.
As described in the Material and Methods sections $CD11b^{hi}CD14^{hi}$ monocytes could be isolated from BMMC. However, dot plots or flow cytometry histograms showing the distribution of the purified cell populations are not presented. Moreover, the number of mice involved in these studies is not clearly indicated. It is also not clear, whether the transfused monocytes in groups 1, 2, 3 derived from one or more donors?	• Flow cytometry histograms were added. • In cell therapy to obtain the number of cells needed for the study we used one donor animal to transplant two recipient animals.
To analyze statistically significant differences between the groups of mice the authors used both parametric and non-parametric tests. Considering the relatively low number of mice (n=5 /group, which factors have been involved for selecting the most relevant statistical analysis?	The number of animals used in this study meets the requirements of the Brazilian Guide for the Creation and Use of Animals for Teaching and Research Activities of the National Animal Experimentation Control Council, which follows the 3Rs principle. In this way we chose the use of isogenic mice because they have a more uniform phenotype than heterogenic, allowing a better detection of treatment responses and reducing the number of animals needed.

	Initially, the quantitative data were submitted to the normality test (Shapiro-Wilk). This test was performed to evaluate if the samples show normal distribution. When the P value was greater than 0.05, it was considered that there was a normal distribution among the samples analyzed, and a parametric statistical test (ANOVA test, with Tukey post-hoc) was indicated; When P is less than 0.05, it is considered that there is no normal distribution among the samples, and a non-parametric statistical test (Kruskal-Wallis test, with Dunn post-hoc) is indicated. These considerations will be added to the "statistical analysis" session, on page 11.
What was the reason to select exclusively male mice for testing the effects of the selected parameters?	When we sent the project to the Committee on Ethics in the Use of Animals we requested authorization for the use of males and females. However, due to the availability of the Animal Breeding Center Laboratory, we only received males. We believe that it did not impair our findings, since in previous studies of cell therapy performed by our group and other groups no difference was observed. .
The figure showing IL-1ß concentration is duplicated in Figure 5 (B and C). The figure showing IL-6 concentrations is missing, while IL-6 is mentioned in the Results.	We apologize for the mistake and it is now corrected
It should be noted that proteins, such as cytokines can be present intracellularly upon translation, and also can secrete it by exocytosis. The terms referring to protein expression and production should be clarified throughout the manuscript.	The text was corrected according to referee's suggestion
In the Title and in the Result section of the Abstract the word *'interleucin'* should be corrected to interleukin. In the Methods section it was described that the mice were kept at ± 23ºC. This text needs revision.	• Title and methods were corrected • On page 13 the text was corrected • On page 14 the text was corrected • On page 15 the text was corrected

On Page 13, the sentence 'A trend was also observed decreased IL-23 cytokine levels (Figure 6D). On Page 14, the sentence 'In this regard, some studies have stated that a decrease in the number of α-SMA$^+$ cells in murine model of liver injury treated with BMCs'. This paragraph needs revision. On Page 15, the word 'proinflamatory' should be corrected to *proinflammatory.*	
The list of references should be selected to end up with a focused and relevant list.	The list was corrected according to referee's suggestion