

ANSWERING REVIEWERS

December 25, 2014



Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14908-review.doc).

Title: Activated rat hepatic stellate cells influence Th1/Th2 profile *in vitro*

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 14908

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated.

2 Revision has been made according to the suggestions of the reviewer.

(1) Authors should criticize their approach or recognize the limits of an *in vitro* research.

We have explained the limitation of *in vitro* study in the discussion section, and we will perform some further *in vivo* studies to verify our results and explore the underlying mechanisms.

(2) Please note that a previous study, Muhanna et al (2008) used a *in vitro* and *in vivo* approach, demonstrating that lymphocytes were in proximity to HSCs primarily within the periportal regions, and some were directly attached or engulfed. Such a possible mechanism was not addressed in the discussion. This should be discussed and this reference included.

We have discussed and cited this reference in this article.

(3) Introduction, last line: "anti-fibrogenic role" and TGF-beta. This cytokine is unanimously recognized as fibrogenic. This should be deleted.

TGF- β secreted by Kuffer cells can activated directed hepatic stellate cells to induce the liver fibrosis. Some report suggested that regulatory T cells (Tregs) play an anti-fibrogenic role by secreting IL-10 and TGF- β . I think that Tregs can suppress immune reaction by IL-10 and TGF- β to inhibit the liver fibrosis course. And I have modified this controversial statements in our article.

(4) Materials and Methods: 5th line from the end The sub-cultured cells were positive for GFAP, desmin and ASMA. When HSC are activated, usually after 1week culture they loose GFAP expression.

This has been checked and modified.

(5) The reference given [15] refers to renal fibroblasts. Please review this reference.

I mean we performed immunochemistry methods according to this reference. This reference is dispensable in this article and it has been deleted.

(6) Why was the ratio 1 HSC:20 CD4 chosen? Does this reflects the *in-vivo* reality?

The ratio of HSC: CD4 lymphocytes was not fixed, which changed with the liver disease progress. We choose a ratio according to the *in vivo* condition and by repeated attempt of *in vitro* co-culture in different ratio.

(7) Considering that clonal expansion is characteristic of the T cell subsets, it is difficult to give relevance to the data given in Fig. 1 showing the proliferation in the absence and presence of HSCs.

Also, why CD4 T cells are dying under these conditions?

If there was no non-specific mitogen (such as PHA, ConA and CD3 monoclonal antibody), T cells *in vitro* would not proliferate and generally undergo apoptosis. But under the stimulation of non-specific mitogen, CD4 T lymphocytes cultured *in vitro* will become CD4 T lymphoblasts and rapidly proliferate, which can not express IFN- γ or IL-4. Therefore, we did not add non-specific mitogen during co-culture to avoid that the influence of HSC on CD4 T lymphocytes was disturbed. And we cocultured HSCs and CD4 T lymphoblasts using Millipore cell culture inserts to prevent the mitogen-like effect of HSCs. So CD4 T cells are dying under these conditions.

(8) The effects/actions of galectin 9 need a more in-depth study.

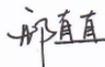
Thank for the suggestion from reviewers, we will do more work in this issue.

(9) The revisions in manuscript have been highlighted in the updated version with green background.

3 References and typesetting were corrected.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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