Sep 5, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: ESPS-4754-review.doc).

Title: PU.1-silenced dendritic cells prolong allograft survival following intestinal transplantation in rats.

Author: Xu Xingwei, Ding Bowen, Zhu Chuanrong, Ji Wu, Li Jieshou

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 4754

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

Part of reviewer 1

(1) If maturation of DCs determines tolerogenecity/immunogenicity why immature DCS did not prolong recipient survival? A discussion on the outcome of the transplantation experiment besides DC maturation would be helpful.

Although immature DCs express low levels of MHC II and determine tolerogenecity, they are not stable in vivo and can easily be stimulated to transform into mature DCs through several signaling pathways. PU.1-silenced DCs are a new kind of stable cells which can also determine tolerogenecity.

We have added the section of transplantation experiment outcome in the second paragraph of Discussion.

(2) Recipient rats that died within three days were regarded as technical failures and excluded from further analysis. It is unclear whether how to tell the difference between technical failure or death due to treatments received prior to the transplantation.

Recipient rats receive DCs injection seven days prior to intestinal transplantation, we have not found this dose of DC cells be lethal according to our observations and the literature data. As rat intestinal transplantation is of complex microvascular techniques, high mortality and hard to conduct, we usually confirm technical failures from autopsy.

(3) Figure 1b showed the reduction in PU.1 expression, but it is unclear how the 85% reduction was quantified.

After strip scanning, we use software to analysis grayscale, we estimate this value as the depth of different strips are obvious.

(4) There should be a comparison for histological changes between the siRNA PU.1 DCs group and the controls, rather than describing only changes in the siRNA PU.1 DCs group as in the last paragraph of the result section.

We have modified and added the section of comparison outcome in the last paragraph of Results.

(5) Semi-mature or semi-matured? Both forms were used in the manuscript. A consistent expression is needed.

We have unified the name "semi-mature".

(6) In the discussion, it was mentioned that the observed effects in the transplantation were consistent with the increase secretion of IL-10, and a decrease in IL-12p70. However, no data were presented about the levels of

IL-10 and IL-12 in the animals.

IL-10 is regarded as an immunosuppressive cytokine. Increase of IL-10 and decrease of IL-12p70 means down regulate the synthesis of inflammatory cytokines and inhibit allogeneic T cells proliferation. We intend to mean that the result of PU.1 gene silence induced tolerance in vivo was consistent with the result of IL-10 and IL-12 change in vitro.

(7) Figure 3. The resolution and contrast of the histological photos are very poor. They are not adequate to show the morphology of lymphocytes. How does the damage (blunting) of villi determined/measured?

We have changed and resubmitted the photos; the damage of villi could be seen from them.

(8) There are several minor language errors in the manuscript.

We have modified and checked the manuscript again.

Part of editor 1

(1). The article was very rough in experiment and presentation.

We have made some modification according the Reviewer's and Editor's suggestions, and added a better detailed description of the experimental results for readers to understand.

(2). Introduction, Line 15; What do cDC and pDC mean?

pDC (plasmacytoid DC, pDC), cDC (conventional DC, cDC). They are both subsets of dendritic cells according to different sources.

(3). Methods, In vitro generation of bone marrow-derived immature DCs, Line 2; Authors wrote "the femoral and tibial were mechanically obtained" What does this sentence mean?

Because the procedure of this experiment has been well known by scientists, we just briefly introduced in our manuscript. (Detailed steps: The rats were killed by cervical dislocation. Then the femur and tibia was removed by aseptic surgery. After discarding the surrounding muscle tissue, we washed the femur and tibia twice with PBS, and kept it into a sterile petri dish on ice).

(4). Authors must show how they select the sequence for siRNA, or should show the reference.

We have added the reference about the selection of sequence for siRNA and primer.

(5). Intestinal transplantation and treatment; Were DCs obtained from Wistar rats or F344 rats?

They were from donor F344 rats. We have added it in the article.

(6). Figure 1A did not have enough information for publication.

After a thoughtful consideration, we decide to delete the figure 1A.

(7). The quality of all figures was poor.

We have checked the figures and provided primitive figure files that can be re-drawn easily.

(8). No figure legend was found.

We have added the figure legend.

3 References and typesetting were corrected

Sincerely yours,

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