Author’s response to reviews

Title: Effects of resveratrol on Th17 cell-related immune responses under tacrolimus-based immunosuppression

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Author’s response to reviews:

<Reviewer 1>
→ no comments.

<Reviewer 2>
→ no comments.

<Reviewer 3>
1. The authors should be mentioned in the introduction the studies on the immunosuppression of tacrolimus on the Th17 derived immune cells are controversial, where there is several studied approve the promise immunosuppression effect of tacrolimus on the Th17 cells.
For example


→ Thank you for your comments. As reviewer comment, we have added the reference in background.

We corrected in the background as below.

On page 3: “Until now, the effect of Tac on regulation of Th17 derived immune cells has been controversial. Previous reports have shown that Tac effectively suppressed Th17 immune responses in experimental models of airway inflammation, osteoclastogenesis and psoriasis, [12-14] but the suppressive effects Tac on Th17 immune responses in kidney transplantation were shown to be somewhat inadequate.[15, 16]”
2. The discussion should be restructure, there are many results in the manuscript controversial with other international studies. The authors should be discussing whey there are difference between their own results and other results, for example:

The authors reported that the Tacrolimus not inhibited the expression and level of IL-17, while the Yago et al., 2012 and Jain et al., 2016, reported the Tacrolimus is potent inhibited the production of IL-17


The authors reported that the resveratrol significantly inhibited the expression and level of IFN-γ, while the Jeong et al., 2014 reported the resveratrol is potent induction IFN-γ.


The authors reported that the Tacrolimus not inhibited the expression and level of IL-22, while the Jain et al., 2016, reported the Tacrolimus is potent inhibited Th-17 to produce IL-22, where reduced by 1.92 fold than positive control.

→ Thank you for your comments. As reviewer comment, we have added the proposed reference in discussion.

We corrected in the discussion as below.

On page 16: “The effects of Tac on regulation of Th17 derived immune cells is controversial. The suppressive effects of Tac on Th17 immune responses has been reported in various experimental studies such as airway inflammation, osteoclastogenesis and psoriasis. [12-14] On the other hand, there are only a small number of animal studies on the effects of calcineurin inhibitors (CNIs) on Th17 responses in transplantation. We previously reported that Tac failed to suppress IL-17 immune responses in kidney transplantation recipients. [15, 16] In this study, we confirmed that Tac alone is not effective in suppressing Th17 immune response. The reason for this is related to the Tac dose. We used lower Tac doses (1ng/ml, 10ng/ml) compared to other experimental studies, based on a cytoxicity test. Higher doses of Tac decreased viability of PBMCs, thus, we chose Tac doses without significant cytoxicity. Furthermore, we considered therapeutic levels used in clinical practice.”

Jeong et al. reported that the resveratrol is potent induction IFN-γ.

Resveratrol has been reported to possess the ability to intervene in multistage carcinogenesis. Resveratrol has been shown to induce apoptosis in LNCaP and DU145 prostate cancer cell lines. But several animal studies have substantiated the anti-inflammatory effects of resveratrol found in vitro.( POULSEN, Morten Møller, et al. Resveratrol and inflammation: Challenges in translating pre-clinical findings to improved patient outcomes. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 2015, 1852.6: 1124-1136.) However, the resveratrol in this paper is not a natural compound. Therefore, the structural formula is different.
A novel resveratrol analogue, HS-1793 does not contain the unstable double bond which resveratrol has. In addition, the position of two of three hydroxyl groups in HS-1793 at the aromatic ring is different from resveratrol. HS-1793 has been shown to inhibit tyrosinase activity more strongly than resveratrol. (HA, Young Mi, et al. 4-(6-Hydroxy-2-naphthyl)-1, 3-bezendiol: a potent, new tyrosinase inhibitor. Biological and Pharmaceutical Bulletin, 2007, 30.9: 1711-1715.)

<Reviewer 4>

The authors examined the additional effects of resveratrol on Th17 cells in the presence of tacrolimus. They reveal that resveratrol can inhibit the induction/differentiation of Th17 cells compared with Th1, Th2, Treg cells in vitro. They also examined activation of p-AMPK and p-mTOR, which showed the contrast results. This combination can prolong the skin graft survival in allogenic combination. The reviewer supposes that this study provides useful information, but several points should be clarified.

1) Why was not the effect of resveratrol alone examined throughout the study? In some experiments, the effect of resveratrol alone is informative.

→ Thank you for your comments. We already performed experiments using resveratrol alone in vitro. As in other studies Resveratrol alone condition did show suppressive effect on the appearance of IL-17, IFN-γ positive T cells. Therefore, our studies focused on combination of Tac to Resv.

2) There is no information how many mice were used in the experiments (Fig. 6a, b, and c).

→ We have added the figure legends
We corrected in the figure legends as below.

On page 27-28: “(a) Kaplan-Meier Survival curve of skin allografts in each group with six animals per group. (b, c) The proportion (%) of IFN-\(\gamma\)+/CD4+ T cells, and IL-17+/CD4+ T cells in isolated spleen cells from each group was measured via flow cytometry in six animals per group”

3) It is difficult to check infiltration of immune cells. Higher magnification should be shown.”

→ We corrected it. We changed magnification x400.

4) References (21, 23, and 25) are incomplete.

→ Thank you for your comment. We have revised the reference.