Receiving Editor

World Journal of Gastroenterology

February 3th, 2024

Dear editor of *World Journal of Gastroenterology*:

Subject: Submission of revised paper 91559.

Thank you for your email enclosing the reviewers' comments. We have carefully reviewed the comments and have revised the manuscript accordingly. Attached is our responses to each comment. Changes have been made to the manuscripts accordingly. We hope the revised version could be considered for publication.

Sincerely,

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Responses to the editors and reviewers

Thanks for the careful and thorough review of our paper. We have answered each of the comments below.

The manuscript by Zhou et al is a timely review of the recent developments in transplantation immunology, with respect to liver. This is short and informative.

1) ……. and a 5.8-fold higher risk of premature death than the general population. —define premature death

Response: The general definition of premature death was death before 70 years old. In the article of Fredrik Åberg (Hepatology. 2015;61(2):668-677), they found that standardized mortality ratios of Nordic LT patients for death before age 75 (premature mortality) was 5.8 (95% confidence interval [CI] 5.4-6.3).

2) The authors need to discuss more on human CAR-Tregs. Othe than HLA-A2, what are the other possible proteins which can be targeted to achieve tolerance?

Response: Thanks very much for the recommendation. In the revised article, we have added more discussion about other targeting proteins of CAR-Tregs therapy like CD83 and GAD65. The CAR-Treg therapy for autoimmune diseases has been conducted in many preclinical and clinical trials and the targeting protein includes Citrullinated vimentin (CV), Myelin oligodendrocyte glycoprotein (MOG), Ganglioside D3 (GD3) and so on. However, since most targeting proteins are expressed by all organ/tissue of the human, they are unable to accumulate the infused CAR-Treg into the liver. Therefore, the optimal candidate target protein used by the CAR-Treg therapy should be mainly expressed in the transplanted liver, which could result in the accumulation of Treg in the liver. Currently, HLA-

A2 is the most common used target protein but protein like CD83, which is mainly expressed in the activated CD4 T cell and dendritic cells could also be a promise target.

3) The failure of tolerogenic DC in liver transplantation also needs more explanation. Is it the inability to generate personalized antigen specific tolerogenic DCs is the major challenge?

Response: Thanks very much for the recommendation. In the revised article, we discussed more about why DCreg infusion failed to induce tolerance in liver transplantation recipients. One possible reason is the short-lived survival of donor DCreg after infusion, which may be killed by the NK cells. Meanwhile, the influence of donor derived DCreg to the immune status of the recipients is unclear. Even though circulating Treg/Teff ratio witnessed increase after DCreg infusion, whether the change is sufficient to induce tolerance is questionable.

4) The usefulness of MSCs in transplantation is far from clear. Most studies show that these MSCs die and cause micro embolus. Even if they survive for few days and secrete some small amount of cytokines, will it have any significant effect for a large organ like liver? The evidences are not convincing. Will it have more effect than an extra dose/short low dose course of immunosuppressant? Is it worth the risk and cost?

Response: Thanks very much for the comment. I totally agree with the reviewer that the MSC therapy is far from translating into clinical practice. One of the main reasons is the identification of optimal culture conditions for ex vivo MSCs since the culture and manufacturing conditions may influence the properties of MSCs. Secondly, the proliferation and longevity of MSCs after infusion is questionable. The generation of antibodies against MSCs and the possible immune rejection in an allogeneic donor after MSCs infusion suggest that MSCs may not be absolutely immune

privileged. Besides, differentiation of MSCs could occur after infusion, which further limited their proliferation. Thirdly, the capacity of migration of MSCs determined whether MSCs could secrete enough cytokines to modulate the recipient's immune function. Therefore, no successful induction of immune tolerance in liver transplantation recipients had been reported so far. Modification to MSCs to improve their stability and secretive function, like genetic modification or three-dimensional culture has been studied in the last decade. Besides, although MSCs infusion is not sufficient to induce tolerance in liver transplantation recipients, many clinical studies had proven its ability to reduce rejection after transplantation. Therefore, the clinical application of MSC therapy could expand to reduce the overall exposure amount of immunosuppressive agents after transplantation, not totally withdraw the immunosuppressant. This is useful for recipients with dysfunction of other organs like kidney. For liver transplantation recipients with renal dysfunction, MSC therapy may reduce the usage of CNI immunosuppressants, which could ameliorate the damage of immunosuppressants to the renal function.

5) The authors are encouraged to make a table condensing all the relevant studies in this direction. This will help the readers to understand the developments quickly.

Response: Thanks very much for the recommendation. In the revised article, we have added Table 1 into the manuscript, which summarized all clinical trials that used cellular therapy to induce tolerance after liver transplantation.