**Response Letter**

**Reviewer's code:** 02446101

Comments from reviewer

**In this review, you clearly summarised how MSCs induce Treg and Breg cells to provoke immunosuppression, which Sufficiently confirmed that MSCs possess a great potential to treat autoimmune diseases. The manuscript provides the readers some innovative and valuable information. Further studies are expected. So, acceptance should be recommended for this manuscript.**

Thank you for recommending our manuscript to be accepted and we appreciate your comments.

**Reviewer's code:** 02446319

Comments from reviewer

**I read your paper, contribute well. Mechanisms involved in Treg and Breg cell induction by MSCs, did well to summarize. If you modified a little more modern and comfortable Figure design, it will be even better.**

Thank you for recommending our manuscript to be accepted and we appreciate your valuable comments. Therefore, we have revised our manuscript according to your suggestion. We have modified our figures to look more contemporary.

**Reviewer's code:** 02398400

Comments from reviewer

**The review by Ma and Chan provides a comprehensive and well-balanced overview of immune effector cell phenotypes and functions, and how MSCs influence the activity of these cells. The authors do a good job of describing cell-based and animal-based studies to support the different proposed mechanisms by which MSC interact with immune effector cells to alter their function. Only a few minor concerns were noted. 1. In the introduction the authors use the phrase "the re-education propensity of MSCs". This term is mis-leading, as "education" of immune cells is typically associated with antigen presentation and clonal deletion. Therefore, the term "re-education" should be replaced. 2. The authors do a good job of pointing out that PGE2 can be anti and pro-inflammatory. Nevertheless, they paint a picture that proteins secreted by MSCs are always therapeutic. Although few negative studies are published, there have been more than a few MSC-based clinical trials that have failed to meet their primary endpoints. A cautious discussion about potential negative effects of cells is warranted. For example, TGF-β1 is pro-fibrotic and therefore its secretion by MSCs in tissues may promote fibrosis. 3. Similarly, the author described the genetic engineering of MSCs as a way to overcome inter-population heterogeneity and poor homing in vivo. However, the latter topics are not discussed in any detail despite the fact that are critical determinants that limit potency. Also, no description of the inherent risks of genetically modifying cells is provided. These topics should be addressed at least in a cursory manner. 3. The authors state that expressed levels in MSCs of adhesion proteins, such as VCAM1 and ICAM, are low under normal conditions. However, these adhesion molecules are known to play fundamental roles in regulating hematopoiesis and HSC trafficking in bone marrow. These authors should clarify the different roles played by these molecules, and that low levels may not influence immune cell function but do regulate hematopoiesis. 4. The manuscript requires editing for English grammar.**

Thank you for the valuable comments. We have taken the comments very seriously. As a result, we have revised our manuscript according to the suggestions.

1. We have replaced the term “re-education propensity of MSCs” by “regulatory-skewing propensity of MSCs”. The revised sentence as
“In addition, the regulatory-skewing propensity of MSCs observed in innate immune system also applies to T and B lymphocytes.”

2,3. We address these comments with a new paragraph before conclusion.

**Safety and concern of MSCs as cellular therapies in patients**

To date, there are nearly 500 ongoing MSCs-based clinical trials. They aim to investigate the effectiveness of MSCs on treating different diseases, including GvHD, diabetes, cardiovascular diseases, hematological diseases and neurological diseases[103]. Although most of these clinical trials reported patients tolerated MSCs infusion and administration well, there are some safety concerns that require caution. During *in vitro* expansion, MSCs can give rise to replicative senescence, which may affect the activity of surrounding healthy cells and therefore, reduce the clinical efficacy. Moreover, although MSCs have low immunogenicity due to the reduced expression of co-stimunlatory receptors and major histocompatibility complex (MHC) class II antigens, *in vitro* stimulation of MSCs with pro-inflammatory cytokines can upregulate MHC class I and class II expression, compromising the hypo-immunogenicity property of MSCs. These problems can be settled by standardizing the isolation, *in vitro* expansion and purification procedures in order to prevent any inconsistency in clinical efficacy. However, therapy involving genetically-modified MSCs may require more attention since there is a potential risk of MSCs becoming carcinogenic after genome editing. Some studies showed MSCs are vanished in a short period of time after infusion while the immunomodulatory effects of MSCs are long-lasting[104]. The risk of carcinogenicity will probably be extremely low in therapy in which MSCs only stay in the body for short duration temporarily. Nonetheless, regenerative medicine requires MSCs to be retained in the body. Increased numbers of studies focused on how to maximize the migration ability, differentiation capacity and survival of MSCs *in vivo* through genetic modification in order to increase the treatment efficacy[105]. In this scenario, extra precaution and thorough understanding of the short-term and long-term effects of MSCs to the human body are necessary before administration to patients.

3. Recent studies revealed that MSCs actually suppress the expression of VCAM1 and ICAM on T cells. Hence, MSCs attenuate T cells infiltration into the CNS. We have added the following sentence in the paragraph of Cell-cell interaction.

"It is noteworthy that MSCs can inhibit the expression of ICAM-1, CXCR3 and -integrin on $CD3^+$ T cell, hence diminish the interaction between T cells and endothelial cells."

- **The manuscript requires editing for English grammar.**

  Grammar has been carefully checked and edited.

**Reviewer's code:** 00503126

Comments from reviewer

**Ma and Chan review mechanisms by which MSCs interact with Treg and Breg cells to modulate immune responses. This is an important area in biomedical research, as multiple centers are pursuing the use of MSCs for clinical use. The paper is a condense, but informative depiction of the field. Some suggestions to improve the manuscript include: 1. Because of the confusion in the Treg field, a recent recommendation was published suggesting a uniform nomenclature (Nature Immunology 14, 307–308 (2013)). It is suggested that the Treg section of this review conform with the published recommendations, wherever possible. 2. In the sentence indicating the "Retroviral viral transfer of FoxP3 to na ïve T cells upregulated the expression of Treg cell-associated genes", it is important to not that FoxP3 only upregulated the expression of a subset of Treg cell-associated genes. 3. In the discussion of Breg cells, there is a statement: "So far, there are several Breg subsets have been identified in mice. They include CD5+CD1dhi B, Tim1+ B cells, and marginal zone B cells". There are subsets within these populations that include Breg cells, but the entire population does not consist of B cells, particularly for MZ B cells. 4. In addition, improvement of grammar and sentence structure would assist greatly in the readability of the manuscript.**

Thank you for the valuable comments. We have taken them very seriously. We have revised our manuscript according to the suggestions.

1. We have unified our terminology according to the publication you recommended (Nature Immunology 14, 307–308 (2013)). $T_{reg}$ cells that derived from thymus are now named $tT_{reg}$ cells. Treg cell that derived from peripheral are now named $pT_{reg}$.

2. We specifically list genes that are upregulated by Foxp3 to avoid any misunderstanding. The sentence is revised as followed:
   "Retroviral transfer of *Foxp3* to naïve T cells ($CD4^+CD25^-Foxp3^-$) can upregulate the expression of some $T_{reg}$ cell-associated genes, including CD25, CTLA-4, GITR and CD103, and the *Foxp3*-transduced T cells were shown to be suppressive."

3. We deleted marginal zone B cells since it may confuse the readers. The statement is revised as followed:
   "So far, several B cell subsets have been identified as $B_{reg}$ cells in mice. They are $CD5^+CD1d^{hi}$ B (B10) cells and $Tim1^+$ B cells [75-77]."

- **In addition, improvement of grammar and sentence structure would assist greatly in the readability of the manuscript.**
  Grammar and sentence structure have been carefully checked and edited.

**Reviewer's code:** 02446219

Comments from reviewer

**The authors have summarized immunomodulatory properties of MSCs. Overall, The review is very well written and at the same time comprehensive. It is very informative to readers working in the field, and very instructive to readers working outside of the field. I regard this manuscript is worth publishing**

Thank you for recommending our manuscript to be accepted and we appreciate your comments.