Author's response to reviews

Title: Human adipose-derived mesenchymal stem cells attenuate collagen antibody-induced autoimmune arthritis by inducing expression of FCGIIB receptors

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Author's response to reviews:

Reviewer number :1

- The effect of ASCs on the expression of FCGR on macrophages would have been more relevant if performed on macrophages isolated from mouse and then cultured in vitro rather than using a cell line. The effect of activation on the expression of FCGR on macrophages is not evaluated, nor the possible induction of a switch from M1 to M2 macrophages.

Answer) As you commented, it would be better to perform experiment with primary cells. However, it was difficult to isolate sufficient amount of primary cells which was equivalent to ASCs. Our goal was to investigate systemic effect of ASC rather than primary cells. We injected ASC intraperitoneally. So, we chose peritoneal cells as primary contact cell with injected ASC. Macrophage is known to be a major population of peritoneal cells. RAW264.7 cell was used as an alternative macrophage cell line in this experiment. M1 and M2 switch is interesting point of view, we are going to prove this at next series of further research.

- The effect of ASC administration in vivo on the expression of FCGR has been tested on peritoneal macrophages. It would be more relevant to isolate macrophages from lymphoid organs or synovium where inflammation is located in this model. Is there a direct correlation between peritoneal and synovial inflamed macrophages?

Answer) We did not trace the migration of MSC to joint organ. If we could
observe the migration and existence of MSC at joint, macrophage isolation from joint may be more correct procedure. Our study limitation was to observe the anti-arthritic effect of intraperitoneally injected MSC. At further experiment, we plan to compare peritoneal and synovial inflamed macrophage.

- The relevance of evaluating various proinflammatory adipokines in respect to FCGR expression is not clearly explained. The present model is not a model of obesity-induced/associated RA. Please explain.

Answer) We agree that CIA model is not obesity-associated RA model. In previous studies, adipokine such as leptin and resistin were reported to be involve in RA (Ref 1, Ref 2) pathophysiology. In our study, level of leptin and resistin decreased in ASC-treated group. We would like to know serum level of adipokines, because ASC was isolated from adipose tissue.

Reference


- In the result section, end of first paragraph, the authors say that “ASCs were injected after arthritis symptoms arose”: at day 4, the incidence is 0, no clinical signs are detected. This is therefore a more preventive than curative approach since the cells were injected 3 days post-immunization and not post-onset.

Answer) We were sorry to give confusion in describing the result. CAIA usually develops arthritis after LPS boosting. LPS boosting abruptly increases arthritis score for short period. After LPS boosting, it is regarded as to concomitantly start RA pathophysiology even if without symptomatic phenomenon (Ref 3). We performed LPS boosting 3 days after immunization, so we described as “post-group” on the group of 4 day injection. We changed the confusing sentence from “ASCs were injected after arthritis symptoms arose” to “ASCs were injected after LPS boosting.”

Reference


Reviewer number: 2

1. use SCID mice induce CAIA in that mice to see if they get th esame results and aame effects from stem cell therapy..

Answer) SCID mice is very difficult to induce arthritis because they are lack of immune system. We usually use collagen induced arthritis (CIA) or collagen antibody induced arthritis (CAIA) model. Pathophysiology of CIA is composed of antigen (Ag) presentation, T cell activation, B cell activation, and antibody (Ab)
production. CAIA is evoked directly by Ag-Ab immune complex formation without help of T and B cells. We would like to reveal pure anti-arthritic effect of MSC in CAIA model. CAIA well simulates arthritic mechanism of macrophage and immune-complex.

2. They should look the effects of stem cells in present experiments on Treg cell changes, Th17 cells changes, Th1 cell changes in addition to their FC gamma receptors, before making such big conclusions. Authors should describe the surface markers of the stem cells they used. Need to describe it briefly, how they produced them and characterize them.

Answer) CAIA mice does not use T cells in arthritis development. (Ref 4). Macrophage and immune complex formation is most important pathologic process in CAIA. That’s why we did not observe Treg, Th17 and Th1. We tried to reveal the MSC action mechanism on basis of the fact that immune complex binds macrophage via FcR.

Reference