Synovial Cell
Induce IL-2 Non-responsiveness to Lymphocytes in Allogeneic Immune Response
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Introduction: Synovial-derived stem cells have great potential for cartilage and meniscus repair. Previously, we showed that mesenchymal-lineage cells derived from various tissues including cartilage, synovium and meniscus failed to stimulate allogeneic responses, and they have the same surface molecular pattern as stem cells derived from bone marrow (1).
Also, we showed previously articular chondrocytes had not only immune privilege but also immune modulatory function and they induced IL-2 non-responsiveness to allogeneic lymphocytes (2).
The purpose of this study was to explore whether human synovial cells have an immune modulatory function like articular chondrocytes do and whether they induce IL-2 non-responsiveness.

Methods:
Cell Culture: Human synovial cells (SCs) were isolated from 12 osteoarthritis knee joints aged 64-84 when total arthroplasty was performed under the informed consent. IRB was approved. SCs were cultured and expanded in DMEM with 10% FBS. SCs were used at the passage 2 or 3.
Mixed lymphocyte reaction (MLR):
Peripheral blood mononuclear cells (PBMCs) were collected after Histopaque-1077centrifugation of whole blood from healthy donors. In order to prepare MLR culture, 5x10⁵ irradiated (15 Gy) stimulator PBMCs were co-cultured with 5x10⁵ responder allogeneic PBMCs harvested from individual donor in RPMI1640 medium supplemented with 10% FBS for 4 days for triplicate samples.
Cell proliferation assay: First, in order to immune modulatory function of SCs, irradiated (30 Gy) third-party SCs were plated in MLR in triplicates onto 96-well round bottom plates at 0, 4x10³, 2x10⁴ or 1x10⁵ cells /well. Second, in order to explore the role of IL-2, recombinant human IL-2 (rhIL-2) was added to the each plate at the final concentration of 0, 50 or 500 IU /ml.To measure the proliferation of responder lymphocytes, 1 μCi of 3H-thymidine was added to each well 18 hours before of the end of culture. Results were expressed as counts per minute (c.p.m.) ± S.D.
Cytokine enzyme-linked Immunosorbent assay: To quantify the IL-2 protein and IL-2 soluble receptor α (IL-2 sRα) in the culture, 100 μl of supernatant was taken from the culture at the end of the cell proliferation assay, and the concentration of these proteins was quantified by ELISA.
Activating CD4⁺ T Cells Proliferation Assay: Purified CD14⁻CD4⁺ T cells (CD4⁺ T cells) were isolated using MACS Magnetic cell sorting system (Miltenyi Biotec). 1x10⁵ CD4⁺ T cells were activated with anti-CD3 and -CD28 mAbs poly-clonally, and they were co-cultured with MCs at R/S ratio 1/1 to 25/1 for 4 days. The proliferation of these activated CD4⁺ T cells was measured in the same manner as the MLR assay.
Flow Cytometric Analysis: In order to explore the expression of IL-2 receptor α(CD25), β(CD122), γ(CD132) on activated CD4⁺ T cells in the proliferation assay at R/S ration 1/1, flow cytometric analysis was performed at 24, 48 and 72 hours after the culture.

Results: 1,Third-party SCs were co-cultured with MLR culture for 4 days. Third-party SCs inhibited MLR in a dose-dependent fashion. (Figure 1)
2,Third-party SCs were co-cultured with MLR culture added 0, 50 or 500 IU /ml of rhIL-2 for 4 days. Exogenous rhIL-2 accelerated MLR, whereas it did not restore the inhibitory effect of SCs. (Figure 2)
3,Third-party SCs did not affect a concentration of IL-2 protein in the MLR assay (Figure 3A), while IL-2 sRα protein level was significantly reduced in the culture supernatants (Figure 3B).
4,Allogeneic activated CD4⁺ T cells were co-cultured with SCs. SCs inhibited the proliferation of activated CD4⁺ T cells. (Figure 3A)
5,Allogeneic activated CD4⁺ T cells were co-cultured with SCs at the
ratio 1/1. (Figure 4A) In the flow cytometric analysis, the expression of the IL-2 sRα on activated CD4⁺ T cells co-cultured SCs did not decrease statistically compared with it on activated CD4⁺ T cells cultured alone. (Figure 4B)

**Discussion:** In the present study, third-party SCs inhibited the proliferation of allogeneic antigen activated lymphocytes (Figure 1), and SCs inhibited the proliferation activated CD4⁺ T cells (Figure 4A). These results indicate that SCs have immune modulatory function similar to articular chondrocytes (3,4) and mesenchymal stem cell (MSCs) (5).

IL-2 is produced by T cells and target cells are T cells, B cells and NK cells. IL-2 has the critical role in the mitogenic activity of lymphocytes and T cell proliferation. In the present study, third-party SCs did not reduce IL-2 secretion of allogeneic lymphocytes in ELISA assay and exogenous rhIL-2 could not restore SCs-induced inhibition MLR (Figure 2) similar to articular chondrocytes(2), MSCs(5). These results indicated that SCs induced IL-2 non-responsiveness to allogeneic lymphocytes and inhibited the proliferation of allogeneic lymphocytes exposed to IL-2.

In this study, we also explored the expression of IL-2 receptors on activated CD4⁺ T cells. We found that SC inhibited the expression of IL-2 receptor α(CD25) on the activated CD4⁺ T cells as well as articular chondrocyte did. We also showed that IL-2 sRα protein level was significantly reduced in the MLR culture supernatant. These results might indicate that SCs inhibited the proliferation of allogeneic activated CD4⁺ T cells through IL-2 receptor α cleavage. It was reported that MSCs suppressed T cell activation though CD25 cleavage (6), but articular chondrocytes did not (2).

We assumed that the immune property would be changed after the tissue specific differentiation, while the immunomodulatory functions seems to be preserved in mesenchymal-lineage cells including synovium cells. Clinically, this study might help for the strategy of allogeneic synovial-derived stem cell transplantation.

**Significance:** CONFLICT OF INTEREST The authors have no conflict of interest.

**Acknowledgments:**

**References:**

(1) Abe S., Nochi H. et al., Trans ORS 34:939, 2009
(2) Nochi H., Abe S. et al. Trans ORS P-1022 2009
(Figure 1) X axis indicates the number of SCs and Y axis indicates the c.p.m. *; P<0.01 vs. MLR culture without SCs.
(Figure 2) X axis indicates the number of SCs. Y axis indicates c.p.m. *; P<0.01 vs without any IL-2 added group.
(Figure 3) Concentrations of IL-2 protein (A) and IL-2sRα protein (B) in the culture supernatants of MLR assay were quantified. X axis indicates the number of SCs. Y axis indicates the concentration of the protein (pg/ml). *; P<0.01 vs MLR culture without SCs.
(Figure 4) X axis indicates the number of SCs and Y axis indicates the c.p.m. (A) Positive staining cells were calculated by histograms in flow cytometric analysis. Blue line indicates activated CD4+ T cells and red line indicates activated CD4+ T cells co-cultured with allogeneic SCs. (B)