Human iPSC-MSC-Derived Xenografts Modulate Immune Responses by Inhibiting the Cleavage of Caspases

CHENG-LIN LI, YUN LENG, BIN ZHAO, CHANG GAO, FEI-FEI DU, NING JIN, Qi-ZHOU LIAN, SHUANG-YUE XU, GUO-LIANG YAN, JUN-JIE XIA, GUO-HONG ZHUANG, QING-LING FU, ZHONG-QUAN QI

ABSTRACT

Mesenchymal stem cells (MSCs) negatively modulate immune properties. Induced pluripotent stem cells (iPSCs)-derived MSCs are alternative source of MSCs. However, the effects of iPSC-MSCs on T cells phenotypes in vivo remain unclear. We established an iPSC-MSC-transplanted host versus graft reaction mouse model using subcapsular kidney injection. Th1, Th2, regulatory T cells (Treg), and Th17 phenotypes and their cytokines were investigated in vivo and in vitro. The role of caspases and the soluble factors involved in the effects of MSCs were examined. We found that iPSC-MSC grafts led to more cell survival and less infiltration of inflammatory cells in mice. iPSC-MSC transplantation inhibited T cell proliferation, decreased Th1 and Th2 phenotypes and cytokines, upregulated Th17 and Treg subsets. Moreover, iPSC-MSCs inhibited the cleavage of caspases 3 and 8 and inhibition of caspases downregulated Th1, Th2 responses and upregulated Th17, Treg responses. Soluble factors were determined using protein array and TGF-β1/2/3, IL-10, and MCP-1 were found to be highly expressed in iPSC-MSCs. The administration of the soluble factors decreased Th1/2 response, upregulated Treg response and inhibited the cleavage of caspases. Our results demonstrate that iPSC-MSCs regulate T cell responses as a result of a combined action of the above soluble factors secreted by iPSC-MSCs. These factors suppress T cell responses by inhibiting the cleavage of caspases. These data provide a novel immunomodulatory mechanism for the underlying iPSC-MSC-based immunomodulatory effects on T cell responses.

KEY WORDS. MSC • Immune • Regulatory T cells • T helper cell • Caspase

SIGNIFICANCE STATEMENT

We established an iPSC-MSC-transplanted host versus graft reaction mouse model using subcapsular kidney injection and demonstrated an underlying mechanism for the immunom suppressive effect of iPSC-MSCs through inhibition of the cleavage of caspases, which was inhibited by a series of paracrine factors secreted by iPSC-MSCs. Inhibition of caspases suppressed T cell responses, decreased Th1 and Th2 frequency, and increased CD4⁺CD25⁺Foxp3⁺ regulatory T cells. These data indicate a novel mechanism for the immunomodulation of MSC via regulation of caspases.

INTRODUCTION

Mesenchymal stem cells (MSCs) are defined as a group of progenitor cells capable of differentiating into a number of mesenchymal lineages such as osteoblasts, chondrocytes, myocytes, adipocytes [1, 2], and endothelial cells [3, 4]. Stem cells are the seeds for tissue repair and regeneration, and in addition, the immunomodulatory effects of MSCs make them a promising source for novel therapies [5]. Increasing data show that MSCs negatively modulate immune properties by suppressing the T cell responses [6–8], inhibiting dendritic cell maturation and antigen presentation [9, 10], impairing B cell activation and proliferation [11], reducing the natural killer (NK) cell proliferation and cytotoxicity [12, 13], and promoting the generation of regulatory T cells (Tregs) [14, 15]. Animal studies and preliminary clinical trials have indicated that MSCs are a promising resource for cell therapy, including for a series of immunological diseases, such as graft-versus-host disease [16], arthritis [17, 18], Crohn’s disease [19], and systemic lupus erythematosus [20, 21].

Pluripotent stem cell-derived MSCs, including embryonic stem cell (ESC)-derived MSCs (ESC-MSCs) and induced pluripotent stem cell...
(iPSC)-derived MSCs (iPSC-MSCs), share a similar phenotype as bone marrow MSCs (BM-MSCs) [22, 23]. We previously differentiated iPSCs to MSCs and reported that iPSC-MSCs, when compared to BM-MSCs, showed a similar phenotype but longer life span [22]. Transplantation of iPSC-MSCs not only inhibited both CD4 and CD8 positive T cell subsets [24], but also expressed less HLA-II under IFN-γ stimulation [25]. MSCs isolated from diverse human iPSC cell lines can strongly inhibit the cytokotoxic functions of NK cells [26] and T helper 2 (Th2) cells mediated allergy [27]. Moreover, iPSC-MSCs are more resistant to pre-activated NK cells than BM-MSCs [26]. More importantly, these iPSC-MSCs homed to cancers with efficiencies similar to BM-MSCs but were much less prone than BM-MSCs to promote the epithelial–mesenchymal transition, invasion, stemness, and growth of cancer cells [28]. Therefore, iPSC-MSCs may be useful as a novel alternative stem cell resource.

Antigen-specific CD4+$^+$ T cells have been reported to provide immunological help not only to CD8+$^+$ T cells and B cells, but also to NK cells in mice and nonhuman primates [29–31]. CD4+$^+$ T helper cells differentiate into several diverse subsets to provide host protection from a variety of pathogens [32]. Th1 cells commonly express interleukin (IL)-2 and IFN-γ, and are involved in cell-mediated inflammatory reactions, where these cytokines activate cytotoxic and inflammatory functions [33, 34]. Th2 cells secrete IL-4, IL-5, and IL-13 that provide immunity against extracellular parasites by helping the B cells in antibody production [35]. In an in vitro coculture system, MSCs decrease the secretion of IFN-γ from Th1 cells and increase the secretion of IL-4 from Th2 cells [36]. However, the detailed effects of iPSC-MSCs on T cell phenotypes in vivo remain unclear.

In the present study, we established an in vivo host versus graft reaction (HvGR) model to study the immunomodulatory properties of iPSC-MSCs on T cell responses. Vascular endothelial cells are known to play an important role in the initial step of eosinophil recruitment and activation during the immune and inflammatory responses [37]. Therefore, in this study human umbilical vein endothelial cells (HUVECs) were used as a positive control, and transplanted into the subcapsule of mice with or without iPSC-MSCs. The results indicated that iPSC-MSCs secrete a large amount of cytokines involved in immunomodulation, and the cytokines exhibited an immunosuppressive effect by inhibiting cleavage of caspase 3 and caspase 8 in T cell.

Establishment of HvGR Model and Cell Transplantation
To establish the HvGR model, 24 female C57BL/6 (H-2K$^d$) mice (Slac Laboratory Animal Co. Ltd, Shanghai, China, www.slaccas.com) were randomly divided into four groups: Control, HUVECs, MSCs, and HUVECs-MSCs together (cografts). Cell grafts (2 \times 10^6 MSCs in 100 μl PBS) were prepared and transplanted into the mice by kidney sub-capsular injection with modification as described previously [38, 39]. The mice in the cograft group were transplanted with 1 \times 10^6 HUVECs and 1 \times 10^6 MSCs in 100 μl PBS together; the Control group mice were transplanted with an equal volume of PBS. For graft survival detection, cells were pre-stained with PKH26 (Sigma Aldrich, St. Louis, www.sigmaaldrich.com) before transplantation (in the cograft group, only HUVECs were stained) according to the manufacturer’s protocol. One week post-transplantation, grafts, serum, spleen, and kidney were harvested from the recipient mice for the next experiments.

Pathological Analysis
The kidney and the cell grafts were resected from the recipient mice 1 week post-transplantation. Tissues were fixed in 4% paraformaldehyde, embedded in paraffin, cut into 5-μm sections, stained with hematoxylin and eosin, and examined by microscopy. Infiltrating inflammatory cells of each section were counted at ×3 magnification by three independent pathologists. Grafts with PKH26-labeling cells were collected, embedded under optimal cutting temperature (OCT, Sakura Finetek USA Inc., www.sakura-americas.com), cut into 5-μm sections, and examined under the FV1000 confocal laser scanning microscope (Olympus, Tokyo, Japan, www.olympus-global.com). The spleens from each group were collected and the protein levels of Cleaved-caspase 8, Cleaved-caspase 3, Cleaved-Poly (ADP-ribose) polymerase (PARP), cyclin D1, cyclin D3 and P27 were detected using Western blot.

Splenocyte Isolation and T Cell Purification
As described previously [39], T cells were purified from the isolated splenocytes by negative isolation with nylon wool columns (Wako, Richmond, VA, www.wakousa.com) at ~90%–95% purity, and viability > 80% (data not shown). T cells or splenocytes from four groups of mice were detected for Th1, Th2, Treg, or Th17 using flow cytometry (FCM) (Beckman Coulter, Inc., www.beckmancoulter.com).

T Cell Proliferation Assays
T cells from four groups of mice were seeded in 96-well plates (1 \times 10^5/well) and cultured in RPMI 1640 plus 10% FBS with or without 2.5 μg/ml concanavalin A (ConA) (Sigma Aldrich, St. Louis, www.sigmaaldrich.com) to evaluate cell proliferation using BrdU cell proliferation assay kit (Roche Diagnostics, Indianapolis, IN, www.roche.com) and MTT reagent [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma Aldrich, St. Louis, www.sigmaaldrich.com). Furthermore, T cells (1 \times 10^5/well) from normal mouse spleen were collected and cultured with HUVECs (1 \times 10^6/well), iPSC-MSCs (1 \times 10^5/well), and the HUVEC-iPSC-MSCs mix (1 \times 10^5/well) under the stimulation of ConA in 96-well plates for 3 days. The optical density values were measured at 450 nm and 570 nm (reference wavelength at 690 nm) for the BrdU and MTT assays, respectively, using a
microplate reader (Model 680 reader; Bio-Rad Laboratories, Hercules, CA, www.bio-rad.com). In addition, the cell trace carboxyfluorescein succinimidyl ester (CFSE) cell proliferation kit (Molecular Probes, Invitrogen, Eugene, OR, www.thermofisher.com) was used to detect T cell proliferation for trans-well experiments according to the manufacturer’s instructions as previously described [39]. HUVECs (1 × 10^5/well), iPSC-MSCs (1 × 10^5/well), and the HUVEC-iPSC-MSCs mix (1 × 10^5/well) were cultured in a transwell above the T cells (1 × 10^5/well) for 72 hours. Each measurement was performed in triplicate.

Coculture of T Cells or Splenocytes with HUVECs, iPSC-MSCs, and the HUVEC-MSCs Mix

T cells or splenocytes were isolated from normal mouse spleens and directly detected for Th1, Th2, Treg, or Th17 using FCM. In addition, the T cells or splenocytes (1 × 10^5/well) were cultured in 96-well plates with HUVECs (1 × 10^5/well), MSCs (1 × 10^5/well), HUVEC-MSCs mix (1 × 10^5/well), or with indicated stimuli for 3 days. The cells were stained with the CD4, CD25, and Foxp3 antibodies for Treg evaluation using FCM. Moreover, the cells were stimulated with phorbol myristate acetate (PMA, 0.05 µg/ml), ionomycin (1 µg/ml), and brefeldin A (BFA, 3 µg/ml) (All from Sigma Aldrich, St. Louis, www.sigmaldrich.com) for 6 hours before the staining, then stained for CD4, IL-4, IFN-γ, or IL-17A and detected by FCM. The cells were also collected for quantitative real-time PCR (qRT-PCR) and the supernatant was used for examination of IL-2, IL-4, IL-17A, TGF-β, IL-10, or MCP-1 levels.

In order to examine the source of TGF-β and IL-10 produced by MSCs or T cells, T cells from normal mouse spleens were cocultured with MSCs for 3 days, then T cells were removed to another new plates and T cells and MSCs were independently cultured for additional 24 hours. The supernatant was collected to examine the levels of TGF-β and IL-10 using ELISA.

The Treatments of Caspase Inhibitors and Recombinant Proteins

T cells from normal mice spleens were cocultured with HUVECs or stimulated with ConA (2.5 µg/ml), and treated with the caspase inhibitor Z-VAD-FMK (Selleckchem, Huston, TX, USA, www.selleckchem.com), caspase 3 inhibitor Z-DEVD-FMK, and caspase 8 inhibitor Z-IETD-FMK (R&D Systems, Minneapolis, MN, www.rndsystems.com) (all used at a concentration of 25 µM). Th1, Th2, Th17, Treg phenotypes were determined by FCM. Foxp3 mRNA levels were investigated using qRT-PCR. Moreover, T cells from normal mice spleens were stimulated with ConA (2.5 µg/ml), and treated with recombinant cytokines of TGF1/2/3, IL-10, MCP-1, or IL-2 (all 100 ng/ml, R&D Systems, Minneapolis, MN, www.rndsystems.com) for 3 days. Th1, Th2, and Treg phenotypes were determined by FCM. Cleaved-caspase 3 and Cleaved-caspase 8 levels were evaluated using Western blot.

Enzyme-Linked Immunosorbent Assay (ELISA)

Cytokines in serum of recipient mice from different groups and the supernatant for cell culture were detected by ELISA. ELISA was performed using commercially available kits (eBioscience, San Diego, CA, www.thermofisher.com) to detect the concentrations of mouse IL-2, IL-4, IL-10, IFN-γ, TGF-β, and IL-17A, and human IL-10, TGF-β, and MCP-1. For the TGF-β assay in the supernatant, the cells were cultured in serum-free medium and the level of TGF-β in the supernatant was determined using ELISA in accordance with the manufacturer’s instructions.

FCM Analysis

The splenocytes and T cells from the recipients (1 × 10^6 cells/100 µl) or coculture system were stained with fluorescently labeled antibodies according to the manufacturer’s instructions. For intracellular cytokines staining, cells were stimulated with PMA (0.05 µg/ml), ionomycin (1 µg/ml) and BFA (3 µg/ml) (All from Sigma Aldrich, St. Louis, www.sigmaldrich.com) for 6 hours before staining. Antibodies used for FCM analysis were FITC-anti-CD4 (GK1.5), PerCp-anti-CD4 (RM4-5), APC-anti-IL-4 (11B11), PE-anti-IFN-γ (XMG1.2), APC-anti-CD25 (PC61.5), FITC-anti-IL-17A (eBio1787), PE-anti-Foxp3 (FJK-16s, ebioscience, San Diego, CA, www.thermofisher.com) and respective isotype controls (all except Foxp3 from BioLegend, San Diego, CA). Data were collected using FACS (Beckman Coulter, Inc., www.beckmancoulter.com) and analyzed using FlowJo software (Tree Star Inc. Ashland, OR, www.flowjo.com).

RayBio Human Label-Based Antibody Array and GO Oncology Analysis

To study the metabolites of HUVECs and iPSC-MSCs, cells were cultured in the same serum free medium (HG-DMEM:DME/F12 = 1:1) and cellular factors for 24 hours. Cell supernatants were harvested for use in the RayBio Human Label-based Antibody Array (L-Series 507: Label-based Human Antibody Array 1-Glass Slide, AAH-BLG-1–2, Raybiotech, , Inc., Norcross, GA, USA, www.raybiotech.com), in which 507 proteins were detected. Antibody array and GO oncology analysis was performed and analyzed by KangCheng Bio-tech (KangCheng Bio-tech, Shanghai, China, www.kangchen.com.cn). The TMEV software was used to plot the antibody array data.

Moreover, HUVECs and MSCs were collected for qRT-PCR of galectin 3, IL-10, TGF-β1, TGF-β2, and TGF-β3. The levels of TGF-β, IL-10, MCP-1 in the supernatant of HUVECs and MSCs were detected by ELISA.

Western Blots

The proteins from spleens in recipient mice and T cells stimulated with different factors were prepared in RIPA lysis buffer supplemented with protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN, http://www.roche.com). The protein lysates were resolved on a 6%–12% Bis-Tris gel and then transferred onto a PVDF membrane (Roche Diagnostics, Indianapolis, IN, http://www.roche.com). Membranes were probed with specific antibodies, detected with HRP-linked secondary antibody (Thermo Scientific, Pierce, Rockford, IL, www.thermofisher.com), and visualized with the ECL reagent (Thermo Scientific Pierce, Rockford, IL, www.thermofisher.com). Primary antibodies used for Western blotting were anti-cleaved-caspase 3, cleaved-caspase 8, cleaved-PARP, cyclin D1, cyclin D3 and P27 (all from CST, Beverly, MA, www.cellsignal.com), beta-actin (Multisciences, Hangzhou, China, www.liankebio.com) was served as control. Densitometric analysis of the immunoblots was performed using GenoSens Software (GenoSens, Shanghai, China, www.clinx.cn).

Quantitative Real-Time PCR (qRT-PCR)

Total RNA was isolated from the splenocytes of recipient mice, cultured T cells in different situation, or human HUVECs and MSCs using TRIzol (Life Technologies, Carlsbad, CA, www.thermofisher.com).
Reverse transcription and quantitative RT-PCR were performed using commercially available reagents (Toyobo, Osaka, Japan, www.toyobo-global.com). PCR amplification was performed to detect the relative mRNA levels of murine IL-2, IL-4, IL-10, IFN-γ, Foxp3, and TGF-β1 and human IL-10, galectin 3, TGF-β1, TGF-β2, and TGF-β3 using the StepOne Real-Time PCR System (ABI, Foster City, CA, www.thermofisher.com). Cross-species specific β-actin was used as a normalizing control. Each reaction was carried out in triplicate. Calculation was performed using the 2−ΔΔCT method. The primer sequences used for the qRT-PCR are listed in the Supporting Information (Supporting Information Table 1).

Statistical Analysis
Data were expressed as the mean ± SD and analyzed using SPSS16.0 software (SPSS, Chicago, IL, USA, www.ibm.com).

Statistical analysis for the PCR data in two groups was performed by Student’s t test. Multiple comparisons more than two groups were examined for statistical significance with one-way analysis of variance followed by LSR. A probability value of p < .05 was considered statistically significant. Statistically significant differences are indicated in the figures with the indicated superscript letters.

RESULTS
Low Immune Inflammation with Human Xenografts of iPSC-MSCs in an HvGR Model
To investigate the immunomodulation of iPSC-MSCs, an in vivo HvGR model was established by transplanting HUVECs...
into the subcapsular region of the kidneys of C57BL/6 mice with or without iPSC-MSCs (Fig. 1A). Graft survival was tested by pre-labeling the HUVECs or iPSC-MSCs with dyestuff PKH26 (red) before transplantation, and photographed using the confocal laser scanning microscope. One week after transplantation, less PKH26-labeled cells were found in the mice of the HUVEC group. However, the iPSC-MSCs -transplanted group and HUVEC cotransplantation group displayed better survival with a stronger PKH26 signal and more cells detected (Fig. 1B). Furthermore, the grafts from the HUVEC group were filled with infiltrating inflammatory cells, but the iPSC-MSCs group and cograft group showed less infiltrating inflammatory cells (Fig. 1C). These data suggest that there was low immune inflammation with iPSC-MSCs transplantation, and iPSC-MSCs inhibit the immune response that is induced by HUVEC transplantation. Therefore, the iPSC-MSCs may play a role in the immunomodulatory response.

iPSC-MSCs Modulate T Cell Response and the Secretion of Inflammatory Factors

Splenocytes isolated from the recipient mice of four groups were used for the proliferation assay. HUVEC-transplanted group showed significantly increased OD values in the BrdU and MTT assays compared to the Control group. However, there were lower OD values in the iPSC-MSC group and in the cotransplanted group in comparison to those in the HUVEC group, although the values in cotransplanted group were still higher than those in iPSC-MSC group (Fig. 2A, 2B). We next analyzed the levels of CD4<sup>+</sup>IFN-γ<sup>+</sup> primed Th1 cells, CD4<sup>+</sup>IL-4<sup>+</sup> primed Th2 cells, and CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cells in the spleens after transplantation. The percentages of Th1 and Th2 cells in the HUVEC group were significantly increased compared to Control group (Fig. 2C). A significant decrease in the proportions of Th1 and Th2 cells was observed in the iPSC-MSC and cotransplanted groups (Fig. 2C). In addition, the iPSC-MSC group and cotransplanted group also showed an increase in the CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg population compared to that of the Control and HUVEC groups (Fig. 2D). We next examined the cytokines about Th1, Th2, and Treg in the serum. Similarly, the levels of pro-inflammatory factors IL-4, IFN-γ, and IL-2 increased in HUVEC group and decreased in the iPSC-MSC and HUVEC+iPSC-MSC cotransplanted groups Fig. 2E. In contrast, low levels of anti-inflammatory factors IL-10 and TGF-β which mainly produced by Treg were observed in Control and HUVEC groups, and higher levels were observed in the iPSC-MSC and HUVEC+iPSC-MSC groups (Fig. 2E).

Next, we cocultured T cells from normal mice with HUVECs, iPSC-MSCs or the HUVEC-iPSC-MSC mix to further confirm the above data in Figure 2. Similarly, there were higher Th1 and Th2 levels in the HUVEC group compared to those in the Control group. However, the Th1 and Th2 levels were significantly reduced by iPSC-MSCs in the coculture system, especially the percentage of Th2 cells (Fig. 3A). Interestingly, the proportion of CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs was decreased in iPSC-MSCs and the HUVEC-iPSC-MSC mix (Fig. 3B). We repeated the similar experiments by coculturing splenocytes with iPSC-MSCs and the HUVEC-iPSC-MSC mix and found more CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs in HUVEC and iPSC-MSC culture, and especially in HUVEC-iPSC-MSC mix coculture system (Fig. 3C). It may because there were dendritic cells in the splenocytes which may regulate Treg in the presence of MSCs.

Because Tregs have more important relationship with Th17 cells [40] and previous papers reported that MSCs have immunomodulation on Th17 cells [41], we analyzed the Th17 population in the T cell culture system. As expected, iPSC-MSCs and the iPSC-MSC-HUVEC mix significantly increased the proportion of Th17 cells compared to that of the Control and HUVEC groups (Fig. 3D), which was in accordance with previous findings [41]. Next, we studied Th1, Th2, Th17, and Treg primed transcriptional factors T-bet, GATA3, RORγt and Foxp3 in T cells. Compared to the HUVEC group, iPSC-MSCs did not affect GATA3 but upregulated T-bet, RORγt and Foxp3 mRNA expression (Supporting Information Fig. 1). Moreover, pro-inflammatory cytokines such as IL-2 and IL-4 were inhibited in the iPSC-MSC and HUVEC-iPSC-MSC mix groups compared with those in the HUVEC group, but IL-17A levels were increased, which was consistent with the data above (Fig. 3E). Therefore, our data indicated that the iPSC-MSCs decreased the subset of Th1 and Th2 cells, decreased the pro-inflammatory factors and promoted the expression of anti-inflammatory cytokines.

The Roles of Caspases in the Effects of iPSC-MSCs on T Cell Responses

Based on our observations, we conducted further studies to examine the signaling pathways involved in the immune responses using mouse splenocytes from different groups. Caspase activation is required for T cell proliferation; specifically, caspase 8 is known to play an essential role in T-cell homeostasis and T-cell-mediated immunity [42]. Reduced activation of caspase 8 is paralleled by decreased T cell survival [43]. PARP was the first caspase 3 substrate identified during apoptosis [44], and defects in PARP result in declined T cell proliferation [45]. We found that transplantation of HUVECs induced increased expression of Cleaved-caspase 8, Cleaved-caspase 3, and Cleaved-PARP as well as cell cycle proteins cyclin D1 and cyclin D3, but downregulated the expression of P27 (Fig. 4A). However, transplantation of iPSC-MSCs or combined with HUVECs decreased the cleavage of caspase 8, caspase 3 and PARP, cyclin D1, and cyclin D3, but increased P27, compared to that observed for the HUVEC group (Fig. 4A). We next investigated the T cell proliferation with ConA stimulation under the treatment of total caspase and caspase 8/3 caspase 3 inhibitors. We found that inhibition of total caspase and caspase 8, but not caspase 3 led to impaired T cell proliferation (Fig. 4B). Interestingly, suppression of any one of caspase 3, caspase 8, or total caspase significantly decreased the proportion of Th1 and Th2 cells (Fig. 4C, Supporting Information Fig. 2), and increased the CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg ratio upon stimulation with ConA (Fig. 4D, Supporting Information Fig. 2). Additionally, Foxp3 mRNA expression was increased by caspase inhibitors (Supporting Information Fig. 3). We also examined whether caspase exerts its effect on T cells anergy, and found no difference between the caspase inhibitor groups and the combination of caspase inhibitor with IL-2 groups (Fig. 4B). In addition, we studied the effect of caspase inhibitors on the Th1, Th2 subsets for T cells cocultured with HUVECs, and on the Tregs for splenocytes cocultured with HUVECs. Addition of caspase inhibitor declined both the Th1 and Th2 proportion (Fig. 4E) and increased Th17 (Fig. 4E), Tregs (Fig. 4F). These results suggest that iPSC-MSCs may modulate T cell subsets by suppressing the activity of caspases.
Figure 2. The effects of induced pluripotent stem cell-mesenchymal stem cells on T cell responses and the expression of inflammatory factors after subcapsular kidney transplantation. The splenocytes were isolated from recipient mice 7 days after transplantation. (A–B): The proliferation of recipient splenocytes was examined by BrdU (A) and MTT (B). (C–D): Proportions of CD4^+ IFN-γ^+ Th1 cells in lymphocytes (C), and CD4^+ CD25^+ FoxP3^+ T cells in CD4^+ T cells (D) were analyzed by flow cytometry. (E): Cytokines in serum of recipient mice from different groups were detected by ELISA. Data are shown as the mean ± SD from three independent experiments. *, p < .05; **, p < .01; ***, p < .001 versus Control group; #, p < .05; ##, p < .01; ###, p < .001 versus HUVEC group. Abbreviations: HUVECs, human umbilical vein endothelial cells; MSC, mesenchymal stem cell; Treg, regulatory T cells.
Soluble Factors Involved in the Immunomodulation of iPSC-MSCs

To explore the role of soluble factors and cell-cell contact in the effects of MSCs suppressing immune responses, T cells were purified from normal mouse spleens and cocultured with HUVECs, iPSC-MSCs, or the HUVEC-iPSC-MSC mix, which were separated by transwell. The CFSE detection showed that T cell proliferation was lower in the treatment of iPSC-MSC or HUVECs-iPSC-MSC mix compared to T cells alone or with HUVECs. The flow cytometry assay showed that the proportions of Th1 (CD4+ IFN-γ+ T cells in lymphocytes) and Th2 cells (CD4+ IL-4+ T cells in lymphocytes) were suppressed in the presence of iPSC-MSCs or HUVECs-iPSC-MSC mix compared to T cells alone or with HUVECs. The ELISA assay showed that the levels of IL-2, IL-4, and IL-17A in the supernatant were also lower in the presence of iPSC-MSCs or HUVECs-iPSC-MSC mix compared to T cells alone or with HUVECs. All data are shown as the mean ± SD from three independent experiments. Abbreviations: HUVECs, human umbilical vein endothelial cells; MSC, mesenchymal stem cell; Treg, regulatory T cells.
Figure 4. The roles of caspases in the effects of induced pluripotent stem cell-mesenchymal stem cells on T cell responses. (A): Spleens from mice (n = 3 in each group) were collected for Western blot analysis. T cells (B–E) or splenocytes (F) from spleen of normal mice were used for the experiments. (B): T cell proliferation with the stimulation of ConA with or without treatments of caspase inhibitors and/or IL-2. *, p < .05 versus ConA group; #, p < .05 versus ConA + IL-2. (C–D): Flow cytometry assay for the proportion of Th1 (CD4⁺ IFN-γ⁺ T cells in lymphocytes) and Th2 cells (CD4⁺ IL-4⁺ T cells in lymphocytes) (C) and Treg (Foxp3⁺ CD4⁺ CD25⁺ T cells in CD4⁺ T cells) (D) induced by ConA with or without caspase inhibitors. **, p < .01; ***, p < .001 versus Control group of T cells only; #, p < .05; ##, p < .01; ###, p < .001 versus ConA group. (E–F) Flow cytometry assay for the proportion of Th1, Th2, and Th17 (E) and Treg (CD4⁺ CD25⁺ Foxp3⁺ T cells in CD4⁺ T cells) (F) in the coculture of HUVECs and T cells (E) or splenocyte (F) with or without caspase inhibitors. **, p < .01; ***, p < .001 versus T cell or splenocyte groups; #, p < .05; ##, p < .01; ###, p < .001 versus HUVEC group. Cas i: total caspase inhibitor Z-VAD-FMK; casp 3i: caspase-3 inhibitor Z-DEVE-FMK; casp 8i: caspase-8 inhibitor Z-IETD-FMK. Data are shown as the mean ± SD from three independent experiments. Abbreviations: ConA, concanavalin A; HUVECs, human umbilical vein endothelial cells; MSC, mesenchymal stem cell; Treg, regulatory T cells.
Figure 5. Paracrine factors about induced pluripotent stem cell-mesenchymal stem cells (iPSC-MSCs). (A): T cells from normal mouse spleens were separately cocultured with HUVECs, MSCs, and HUVEC-MSC mix by the transwell and T cell proliferation was detected using CFSE. Green line: negative control; red line: T cells labeled with CFSE. (B): The protein array for the supernatant of HUVECs and iPSC-MSCs. The graph shows the heat map for the 276 proteins secreted. (C): qRT-PCR of TGF-β1, TGF-β2, TGF-β3, IL10, and galectin 3 in HUVECs and MSCs. (D): TGF-β, IL-10, and MCP-1 in the supernatant of HUVECs and iPSC-MSCs were assayed by ELISA. *, p<.05; **, p<.01; ***, p<.001 versus HUVEC for (C–D). (E): TGF-β, IL-10, and MCP-1 in the supernatant of coculture of T cells from normal mouse spleen and HUVECs, iPSC-MSCs or the HUVEC-iPSC-MSC mix were assayed by ELISA. **, p<.01; ***, p<.001 versus Control group; #, p<.05; ##, p<.01; ###, p<.001 versus HUVEC group. (F): The levels of TGF-β and IL-10 in MSCs or MSCs cocultured with T cells. The T cells were removed and MSCs were cultured further 24 hours. **, p<.01; ***, p<.001 versus T cells; ###, p<.01 versus MSC. Data are shown as the mean ± SD from three independent experiments but not for (B). Abbreviations: HUVECs, human umbilical vein endothelial cells; MSC, mesenchymal stem cell.
HUEC-iPSC-MSC mix compared to HUEC treatment (Fig. 5A). The data implied that iPSC-MSCs modulated T cell responses at least partly in a paracrine manner. Consequently, we analyzed the paracrine factors in the supernatants from HUECs and iPSC-MSCs using a RayBio Human label-based antibody array, and detected 507 factors. Among the 507 target factors detected in the supernatant, iPSC-MSCs exhibited a 2-fold and 1.5-fold increase in the ability to secrete IL-10, TGF-β, and MCP-1 levels were higher in the iPSC-MSCs and HUECs, respectively. The identified cytokines contained known markers associated with immunomodulation, such as TGF-β1, TGF-β2, TGF-β3, IL-10, and TGF-β3. The mRNA levels of TGF-β1, TGF-β2, TGF-β3, IL-10, and galectin 3 in HUECs and iPSC-MSCs were also examined, and the results were in accordance with the protein array data (Fig. 5C). The protein levels of TGF-β, IL-10, and MCP-1 were further investigated by ELISA. There was more TGF-β, IL-10, and MCP-1 from iPSC-MSCs than those from HUECs (Fig. 5D). We further detected these anti-inflammatory cytokines in the cocultures of T cells with HUECs, iPSC-MSCs or both. Data showed that human IL-10, TGF-β, and MCP-1 levels were higher in the iPSC-MSCS and coculture system than in the HUEC group (Fig. 5E). We next determined whether IL-10 and TGF-β were produced from iPSC-MSCs after cocultured with T cells. We removed T cells, changed the medium and cultured iPSC-MSCs for additional 24 hours. We found that iPSC-MSCS cocultured with T cells secreted more IL-10 compared to iPSC-MSCS only. In addition, iPSC-MSCs after coculture still produced TGF-β (Fig. 5F). These data suggest that iPSC-MSCs produce more paracrine factors which have the potential to inhibit immune response.

iPSC-MSCs Secrete Many Soluble Factors Associated with the Immune Response

To assess the potential function of the secreted factors detected by the antibody array, the proteins were replaced with corresponding genes, which were classified according to GO annotations into the following categories: biological processes, molecular functions, and cellular components. Among the 276 corresponding genes, 247 genes were found to have known biological roles. We observed that there were 137 factors enriched in the immune process, and 108 factors that participated in the immune response. In terms of immune response, 45 factors were enriched in the innate immune response and 22 factors in the adaptive immune response (Supporting Information Table 2). The analysis also showed that iPSC-MSCs have great potential to regulate different immunocytes. There were 17, 37, 5, 3, and 3 factors enriched in B cells, T cells, NK cell, myeloid dendritic cell, and macrophage activation, respectively (Table 1).

Furthermore, 51 factors were found to be enriched in cytokine production, including chemokine production, cytokine production involved in the immune and inflammatory response, and the production of granulocyte macrophage colony-stimulating factor, IFN-γ, IL-10, IL-12, IL-13, IL-17, IL-2, IL-5, IL-6, IL-8, TGF-β, tumor necrosis factor superfamily cytokine, type I interferon, and vascular endothelial growth factor (Supporting Information Table 3). It is worth mentioning that iPSC-MSCs highly expressed 70 soluble cytokine receptors including TLR1, TLR2, TLR3, TLR4, and 14 tumor necrosis factor receptor super family (TNFRSF) members such as TNFRSF3, TNFRSF10A, TNFRSF19, and 6 chemokine receptors (Supporting Information Table 4). The data suggest that iPSC-MSCs display an important role in immunomodulation.

Table 1. GO oncology analysis of immunocytes activation-enriched genes

| GO ID   | Term                        | Count | p value       | GENES                                                                 |
|---------|-----------------------------|-------|---------------|----------------------------------------------------------------------|
| GO:0042113 | B cell activation        | 17    | 1.23623E-08  | TLR4//KIT//BAX//IL10//IL12/VCAM1/TNFRSF13 B/TNFRSF21//IL7//IL21//IL7R//INHBA//SFRP1//TGF1//IL27RA/TNFSA//CCKR5   |
| GO:0042110 | T cell activation        | 37    | 6.52318E-19  | BAX/ITGAL//IL7//IL7R//KIT//LCK/GRB2/CTNNB1//SHH/TNFSA//IL2//IL12/IL12A/IL2//CCL5//VCAM1//IGF1//IGF2//FGF2//FGF2P2//IL12B1//IL27//LFA3//TNFSTF21//IL10//IL20RB//TGFβ1//IL18R1//IL36β//CLC//INS//INTGAM//CC12//TGFBR2//XCL1 |
| GO:0001773 | Myeloid dendritic cell activation | 3    | 0.005671342 | IL10//TGFβ1//TGFβ2                                                 |
| GO:0042116 | Macrophage activation     | 5     | 0.000666099  | TLR2//TLR3//IL1RA//TLR1//TLR4                                     |
| GO:0030101 | NK cell activation        | 5     | 0.004370584  | IL12B2//IL12A//IL21//IL21B//IL18R1                                   |

"GO ID" stands for the ID of gene ontology term. "Term" stands for the name of gene ontology term. "Count" stands for the number of DE genes associated with the listed GOID. "p value" stands for the significance testing value of the GOID. "GENES" stands for the DE genes associated with the GOID.
Figure 6. Induced pluripotent stem cell-mesenchymal stem cells regulate immune response through soluble factors. T cells from normal mouse spleens were cultured in different situations. (A): T cells proliferation under the stimulation of ConA or combination with TGF-β, IL-10, MCP-1, and IL-2. (B): Western blot analysis for cleaved caspase 8 and cleaved caspase 3 in T cells with the treatment of TGF-β1/2/3, IL-10, MCP-1, and IL-2 (i). (ii): Statistical analysis. (C-D): Flow cytometry assay for Th1 (CD4+ IFN-γ+ T cells in lymphocytes) and Th2 cells (CD4+ IL-4+ T cells in lymphocytes) (C) and Treg (CD4+ CD25+ Foxp3+ T cells in CD4+ T cells) and Treg (D) with the treatment of ConA, TGF-β, IL-10, MCP-1, and IL-2. Data are shown as the mean ± SD from three independent experiments. *, p < .05; **, p < .01; ###, p < .001 versus ConA group. Abbreviations: ConA, concanavalin A; HUVECs, human umbilical vein endothelial cells; MSC, mesenchymal stem cell; Treg, regulatory T cells.
MSCs have been widely studied for their promising application in cellular therapy, tissue engineering, and the manufacturing of the biomaterials. iPSC-MSCs share a similar phenotype to BM-MSCs, and can strongly inhibit the cytotoxic functions of NK cells [13] and T cells [27]. Therefore, similar to BM-MSCs, iPSC-MSCs may also exert immunomodulatory effects. However, the in vivo roles of iPSC-MSCs in regulating the immune response still need to be revealed. Currently, studies on the immunomodulatory roles of MSCs are restricted because of lack of useful in vivo animal models. It has been previously reported that utilizing the teratomas formed, the immunogenicity of ipsc and hESC was detected by subcutaneous injection [46, 47]. However, because of limited blood supply, the immune environment below the epidermis in this model differs greatly from the in vivo environment. In addition, caudal vein injection of MSCs is generally used in vivo model to study the transient immune response [48]. But the limitation is that it is difficult to detect the survival and location of injected MSCs. In the present study, we established an in vivo mouse model by subcapsular kidney injection of iPSC-MSCs. This model of MSC transplantation can be easily observed, and it is better to reflect the in vivo immune environment compared to subcutaneous injection.

Using this model, we observed that 1 week post-transplantation, the ipsc-MSC derived grafts had few infiltrated inflammatory cells, and exhibited better survival than the HUVEC-derived grafts. iPSC-MSC transplantation inhibited the proliferation of splenocytes and decreased the proportions of Th1 and Th2 cells, but increased the number of CD4^+CD25^+Foxp3^- Tregs if T cells were cocultured with iPSC-MSC, but increased CD4^+CD25^-Foxp3^- Tregs when splenocytes were cocultured with iPSC-MSC. We previously reported that iPSC-MSCs regulate DCs to regulatory DCs [25]. The splenocytes include DCs and the iPSC-MSCs may upregulate Tregs under the assistance of regulatory DCs. On the other hand, previous study reported that murine MSCs secrete matrix metalloproteinases, which causes cleavage of IL-2 receptor α (CD25) from the surface of activated T cells [49]. It may be the reason that MSCs decreased Treg if only T cells were cocultured with MSCs. We further investigated Th17 levels and IL-17 expression in the coculture of MSCs iPSC-MSCs. As expected, in a coculture of iPSC-MSCs and T cells, the Th17 proportion in iPSC-MSC group was dramatically increased compared to that of the negative control and HUVEC groups. These results were consistent with previous report that the coculture of BM-MSCs increased Th17 in human PBMCs [41]. Additionally, we found that iPSC-MSCs inhibited T cell activation dependent of soluble factors, suggesting that iPSC-MSCs may inhibit T cell responses by paracrine effects.

We further detected the metabolites of iPSC-MSCs and HUVECs, and analyzed the enrichment of differential proteins secreted by the iPSC-MSCs. Among the 276 differential proteins, 137 proteins were enriched in the immune system covering both the innate and adaptive immune responses, hematopoietic or lymphoid organ development, and leukocyte and lymphocyte activation, whereas, 86 proteins that were enriched in cytokine production, including cytokines involved in the immune response, such as IL-1, IL-2, IL-6, and IFN-γ. Some of these cytokines have been reported to be capable of regulating the immune response, such as IL-10, TGF-β1/2/3, galectin3 [50], MCP1 (also XCL1) [48], and TSG-6 [51]. TGF-β is an inhibitor of Th1 differentiation [52, 53] and plays an essential role in maintaining normal immune function. Approximately 2 weeks after birth, mice with a TGF-β1 null mutation (TGF-β1/-/-) exhibit a progressive wasting syndrome, death associated with abnormal thymus and lymph nodes, and enhanced proliferation of splenocytes and lymph nodes cells [54]. TGF-β2 decreases the migration of lymphocytes in vitro and homing of cells into the central nervous system in vivo [55]. Additionally, we found that iPSC-MSCs secreted 70 soluble cytokine receptors, including TLR1/2/3/4, and 14 TNFRSF members such as TNFRSF3 and TNFRSF19 as well as TGF-β receptors. The soluble cytokine receptors act via 4 mechanisms: (1) generation of soluble receptors by proteolytic cleavage to downregulate membrane-bound receptors and prevent signal generation by the ligands; (2) soluble receptors serving as binding proteins to stabilize the ligand in the extracellular space; (3) soluble receptors competing with membrane-bound receptors for the ligand to decrease receptor-mediated signal generation; and (4) soluble receptors associating with non-binding receptor subunits and ligands to confer ligand sensitivity to cells that do not express membrane-bound receptors [56]. Although the exact function of these soluble cytokine receptors is not known, it can be assumed that these soluble cytokine receptors might exhibit immunomodulatory roles. The iPSC-MSCs secreted a large amount of soluble factors; however, we believe that iPSC-MSCs negatively regulate the T cell response via a combined mutual action of soluble factors secreted by iPSC-MSCs.

We also investigated the signaling pathways of caspases involved in the effects of iPSC-MSCs on immune responses. We found that there were lower levels of the cleavage of caspase 3 and caspase 8 in iPSC-MSC-treated group compared to HUVEC group. Caspase activation is a physiological response to TCR activation. This enzymatic cascade is not only involved in induction of cell death, but might also be involved in the early steps leading to lymphocyte proliferation. Selective cleavage of caspase substrates occurs in viable and proliferating cells. Caspase 3 and other downstream caspases are processed in CD3/IL2 stimulated lymphocytes, which can be blocked by the caspase inhibitor zVAD [44]. PARP-1 deficiency significantly alters the expression of genes associated with the immune response (e.g., chemokines), genes involved in the Th1/Th2 balance, and impaired CD3/CD28-induced T cell activation and IL-4 production [45]. T cell proliferation is caspase-dependent, and CD3-induced proliferation and IL-2 production by human T cells are blocked by inhibitors of caspase activity. This is paralleled by
MSCs Inhibit Immune Responses by Suppressing Caspases

CONCLUSION

Overall, our data provide evidence that the administration of iPSC-MSCs inhibits T cell proliferation, Th1/Th2 responses in vivo and in vitro, and regulate Th17, Treg phenotypes. iPSC-MSCs produce a lot of soluble factors in which TGF-β may be important to regulate T cell responses. MSCs may regulate T subset activities via inhibiting cleavage of caspases.

ACKNOWLEDGMENTS

We thank Dr. Xing-Liang Fan for his help with language editing. This work was supported by grants from the Major State Scientific Research Program of China (2012CBA01303), the National Natural Science Foundation of China (31271038, 81671583, 81322012 and 81373174), the key grant from the Science and Technology Foundation of Guangdong (2015B020225001), the National Science Foundation of Fujian Province of China (No.2016J01414). Microarray experiments were performed by KangChen Bio-tech, Shanghai, China.

AUTHOR CONTRIBUTIONS

C.L.L., Z.Q.Q.: conception and design; Q.L.F.: Provision of study material; C.L.L., Y. L., B.Z., C.G., F.F.D., N.J., Q.Z.L., S.Y.X., G.L.Y.: collection and assembly of data; C.L.L., Y. L., C.G., G.H.Z.: data analysis and interpretation; C.L.L., Q.L.F.: manuscript writing; C.L.L, Q.L.F, Z.Q.Q.: final approval of manuscript; J.J.X, Z.Q.Q.: administrative support; Q.L.F., Z.Q.Q.: financial support.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

REFERENCES

1 Blugemann C, Wu L, Petrigliano F et al. Novel aspects of parenchymal–mesenchymal interactions: from cell types to molecules and beyond. Cell Biochem Funct 2013;31:271–280.
2 Chen J, Park H-C, Addabbo F et al. Kidney-derived mesenchymal stem cells contribute to vasculogenesis, angiogenesis and endothelial repair. Kidney Int 2008;74:879–889.
3 Silva GV, Litovsky S, Assad JA et al. Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model. Circulation 2005;111:150–156.
4 Oswald J, Boxberger S, Jørgensen B et al. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells 2004;22:377–384.
5 Daley GQ. The promise and perils of stem cell therapeutics. Cell Stem Cell 2012;10:740–749.
6 Krampera M, Glennie S, Dyson J et al. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood 2003;101:3722–3729.
7 Tse WT, Pendleton JD, Beyer WM et al. Suppression of alloergic T-cell proliferation by mesenchymal stromal cells: implications in transplantation. Transplantation 2003;75:389–397.
8 Glennie S, Sovieiro I, Dyson PI et al. Bone marrow mesenchymal stem cells induce division arrest energy of activated T cells. Blood 2005;105:2821–2827.
9 Jiang X-X, Zhang Y, Liu B et al. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 2005;105:4120–4126.
10 Ramasamy R, Fazekasova H, Lam EW-F et al. Mesenchymal stem cells inhibit dendritic cell differentiation and function by preventing entry into the cell cycle. Transplantation 2007;83:71–76.
11 Coricione A, Benvenuto F, Ferretti E et al. Human mesenchymal stem cells modulate B-cell functions. Blood 2006;107:367–372.
12 Sotiropoulou PA, Perez SA, Gritzapis AD et al. Interactions between human mesenchymal stem cells and natural killer cells. Stem Cells 2006;24:74–85.
13 Spaggiari GM, Capobianco A, Abdelrazik H et al. Mesenchymal stem cells inhibit natural killer–cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood 2008;111:1327–1333.
14 Keating A. Mesenchymal stromal cells: new directions. Cell Stem Cell 2012;10:709–716.
15 Prevosto C, Zancoli M, Canavelli P et al. Generation of CD4+ or CD8+ regulatory T cells upon mesenchymal stem cell–lymphocyte interaction. Haematologica 2007;92:881–888.
16 Le Blanc K, Rasmussen I, Sundberg B et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 2004;363:1439–1441.
17 Zhou B, Yuan J, Zhou Y et al. Administering human adipose-derived mesenchymal stem cells to prevent and treat experimental arthritis. Clin Immunol 2011;141:328–337.
18 Liu Y, Mu R, Wang S et al. Therapeutic potential of human umbilical cord mesenchymal stem cells in the treatment of rheumatoid arthritis. Arthritis Res Ther 2010;12:1.
19 Forbes GM, Sturm MJ, Leong RW et al. A phase 2 study of allogeneic mesenchymal stromal cells for luminal Crohn’s disease refractory to biologic therapy. Clin Gastroenterol Hepatol 2014;12:64–71.
20 Liang J, Zhang H, Hua B et al. Allogenic mesenchymal stem cells transplantation in refractory systemic lupus erythematosus: a pilot clinical study. Ann Rheum Dis 2010;69:1423–1429.
21 Sun L, Akiyama K, Zhang H et al. Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. Stem Cells 2009;27:1421–1432.
22 Lian QZ, Zhang YL, Zhang JQ et al. Functional mesenchymal stem cells derived from human induced pluripotent stem cells attenuate limb ischemia in mice. Circulation 2010;121:1113. U1191.
23 Frobel J, Hemedla H, Lenz M et al. Epigenetic rejuvenation of mesenchymal stromal
cells derived from induced pluripotent stem cells. Stem Cell Reports 2014;3:414–422.
24. Xiong P, Liu Y, Chu P-H et al. iPSC-MSCs combined with low-dose rapamycin induced islet allograft tolerance through suppressing Th1 and enhancing regulatory T-cell differentiation. Stem Cells Dev 24:1793–1804.
25. Sun YQ, Zhang Y, Li X et al. Insensitivity of human iPS cells-derived mesenchymal stem cells to interferon-γ-induced HLA expression potentiates repair efficiency of hind limb ischemia in immune humanized NOD Scid gamma mice. Stem Cells 2015;33:3452–3467.
26. Giuliani M, Oudrhiri N, Noman ZM et al. Human mesenchymal stem cells derived from induced pluripotent stem cells down-regulate NK-cell cytolytic machinery. Blood 2011;118:3254–3262.
27. Fu Q, Chow Y, Sun S et al. Mesenchymal stem cells derived from human induced pluripotent stem cells modulate T-cell phenotypes in allergic rhinitis. Allergy 2012;67:1215–1222.
28. Zhao Q, Gregory CA, Lee RH et al. MSCs derived from iPSCs with a modified protocol are tumor-tropic but have much less potential to promote tumors than bone marrow MSCs. Proc Natl Acad Sci 2015;112:530–535.
29. Kelly MN, Zheng M, Ruan S et al. Memory CD4+ T cells are required for optimal NK cell effector functions against the opportunistic fungal pathogen Pneumocystis murina. J Immunol 2013;190:285–295.
30. Vargas-Inchaustegui DA, Xiao P, Tueru I et al. NK and CD4+ T cell cooperative immune responses correlate with control of disease in a macaque simian immunodeficiency virus infection model. J Immunol 2012;189:1878–1885.
31. Jost S, Tomezsko PL, Rands K et al. CD4+ T-cell help enhances NK cell function following therapeutic HIV-1 vaccination. J Virol 2014;88:8349–8354.
32. Stritesky GL, Muthukrishnan R, Sehra S et al. The transcription factor STAT3 is required for T helper 2 cell development. Immunology 2011;34:39–49.
33. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 1996;17:138–146.
34. Sallusto F, Lenig D, Mackay CR et al. Flexible programs of chemokine receptor expression on human polarized Th 1 and 2 lymphocytes. J Exp Med 1998;187:875–883.
35. Neurath MF, Finotto S Glimcher LH. The role of Th1/Th2 polarization in mucosal immunity. Nat Med 2002;8:567–573.
36. Aggarwal S Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005;105:1815–1822.
37. Imaiuzumi T, Kumagai M, Sasaki N et al. Interferon-γ stimulates the expression of galectin-9 in cultured human endothelial cells. J Leukoc Biol 2002;72:486–491.
38. Xia J, Chen J, Shao W et al. Suppressing memory T cell activation induces islet allograft tolerance in alloantigen-primed mice. Transplant Int 2010;23:1154–1163.
39. Ma P-F, Jiang J, Gao C et al. Immuno-suppressive effect of compound K on Islet transplantation in an STZ-induced diabetic mouse model. Diabetes 2014;63:3458–3469.
40. Weaver CT, Harrington LE, Mangan PR et al. Th17: an effector CD4 T cell lineage with regulatory T cell ties. Immunity 2006;24:677–688.
41. Eljaafar A, Tartelin ML, Aissauoi H et al. Bone marrow-derived and synovium-derived mesenchymal cells promote Th17 cell expansion and activation through caspase 1 activation: Contribution to the chronicity of rheumatoid arthritis. Arthritis Rheum 2012;64:2147–2157.
42. Salmena L, Lemmers B, Hakem A et al. Essential role for caspase 8 in T-cell homeostasis and T-cell-mediated immunity. Genes Dev 2003;17:883–895.
43. Hinshaw-Makepeace J, Huston G, Fortner KA et al. c-FLIPS reduces activation of caspase and NF-κB pathways and decreases T cell survival. Eur J Immunol 2008;38:54–63.
44. Alam A, Cohen LY, Aouad S et al. Early activation of caspases during T lymphocyte stimulation results in selective substrate cleavage in nonapoptotic cells. J Exp Med 1999;190:1879–1890.
45. Saenz L, Lozano JJ, Valdor R et al. Transcriptional regulation by poly (ADP-ribose) polymerase-1 during T cell activation. BMC Genomics 2008;9:171.
46. Zhao T, Zhang Z-N, Rong Z et al. Immunogenicity of induced pluripotent stem cells. Nature 2011;474:212–215.
47. Rong Z, Wang M, Hu Z et al. An effective approach to prevent immune rejection of human ESC-derived allografts. Cell Stem Cell 2014;14:121–130.
48. Akiyama K, Chen C, Wang D et al. Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand/-FAS-mediated T cell apoptosis. Cell Stem Cell 2012;10:544–555.
49. Park MJ, Shin JS, Kim YH et al. Murine mesenchymal stem cells suppress T lymphocyte activation through IL-2 receptor alpha (CD25) cleavage by producing matrix metalloproteinases. Stem Cell Rev 2011;7:381–393.
50. Chen H-Y, Fermin A, Vardhana S et al. Galectin-3 negatively regulates TCR-mediated CD4+ T-cell activation at the immunological synapse. Proc Natl Acad Sci 2009;106:14496–14501.
51. Choi H, Lee RH, Bazaran N et al. Anti-inflammatory protein TSG-6 secreted by activated MSCs attenuates zymosan-induced mouse peritonitis by decreasing TLR2/NF-κB signaling in resident macrophages. Blood 2011;118:330–338.
52. Gorelik L, Constant S Flavell RA. Mechanism of transforming growth factor β-induced inhibition of T helper type 1 differentiation. J Exp Med 2002;195:1499–1505.
53. Lauour Y, Sutterwala FS, Gorelik L et al. Transforming growth factor-β controls T helper type 1 cell development through regulation of natural killer cell interferon-γ. Nat Immunol 2005;6:600–607.
54. Christ M, McCartney-Francis NL, Kulkarni AB et al. Immune dysregulation in TGF-beta 1-deficient mice. J Immunol 1994;153:1936–1946.
55. Fabry Z, Topham DJ, Fee D et al. TGF-beta 2 decreases migration of lymphocytes in vitro and homing of cells into the central nervous system in vivo. J Immunol 1995;155:325–332.
56. Heaney ML, Golde DW. Soluble cytokine receptors. Blood 1996;87:847–857.
57. Kennedy NJ, Kataoka T, Tschopp J et al. Caspase activation is required for T cell proliferation. J Exp Med 1999;190:1891–1896.