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SUPPLEMENTAL INFORMATION

Bone marrow mesenchymal stem cells are major source of IL-7 and sustain colitis by forming the niche for colitogenic CD4⁺ memory T cells

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Supplemental Material and Methods

Generation of MSC. To purify MSC from BM of RAG-1^{-/-}, IL-7^{-/-} × RAG1^{-/-}, we used the Stemcell Technologies (Vancouver, Canada) MesenCult® system as previously described (1). BM cells from compact bone from each group were harvested, and 2.5 × 10⁷ BM cells in 5 ml complete MesenCult® medium, made up of 40 ml MesenCult® MSC basal medium and 10 ml MSC stimulatory supplements, were cultured in a 12 cm dish at 37 °C in 5% CO₂. After 1 wk, nonadherent cells were aspirated and fresh complete MesenCult® medium was added. Thereafter, a half-volume change of complete MesenCult® medium was performed at weekly intervals. Once cells were 80% confluent, they were passaged with trypsin-EDTA. We used MSC after at least the seventh passage, when Sca-1 expression was high and any hematopoietic marker expression

was low. In addition, we did not use MSC after the fortieth passage for *in vitro* or *in vivo* study, because IL-7 expression in IL-7^{+/+} MSC decreased gradually after this time, possibly as a result of gene silencing (Fig. 2A).

Coculture of colitogenic memory CD4⁺ T cells with IL-7^{+/+} or IL-7^{-/-} MSC.

To investigate whether IL-7^{+/+} MSC are sufficient for the maintenance of colitogenic memory CD4⁺ T cells *in vitro*, we performed the following experiment. LP CD4⁺ T cells were obtained from the colon of colitic RAG-2^{-/-} mice previously injected with CD4⁺CD45RB^{high} T cells. These cells were incubated and labeled with CFSE as previously described (2), and 1 × 10⁶ CFSE-labeled colitogenic memory CD4⁺ T cells were cocultured with 80% confluent IL-7^{+/+} or IL-7^{-/-} MSC in complete MesenCult® medium. CFSE-labeled memory CD4⁺ T cells were incubated alone in complete MesenCult® medium as a negative control. Half-volume medium changes were carefully performed in each group so as not to aspirate any cells, and 4 wk later, CFSE expression was checked by flow cytometry.

Intracellular staining of Bcl-2 of the colitogenic CD4⁺ T_{EM} cells cocultured with IL-7^{+/+} or IL-7^{-/-} MSC.

To clarify further the mechanism of maintenance of colitogenic memory CD4⁺ T cells by IL-7^{+/+} MSC, we checked the expression of Bcl-2 in the CD4⁺ T_{EM} cells cocultured with IL-7^{+/+} or IL-7^{-/-} MSC. Colitogenic CD4⁺ T_{EM} cells were obtained and cocultured as described above. Two days later, staining of intracellular Bcl-2 in the recovered cells was performed and analyzed by flow cytometry. For the IL-7 blocking experiment, 1 × 10⁶ CFSE-labeled colitogenic memory CD4⁺ T cells were cocultured with 80% confluent IL-7^{+/+} MSC in complete MesenCult® medium that included 10 µg/ml of anti-IL-7 polyclonal antibody (R&D Systems) or the same concentration of control IgG.

CFSE-labeled memory CD4⁺ T cells were incubated with 80% confluent IL-7^{+/+} MSC as a negative control. Half-volume medium changes with complete medium including anti-IL-7 antibody or control IgG or with medium alone were carefully performed in each group so as not to aspirate any cells, and 4 wk later, CFSE expression was checked by flow cytometry.

MSC suppression assay *in vitro*

First, IL-7^{+/+} MSC and IL-7^{-/-} MSC were cultured in respective well. The next day, CFSE-labeled CD4⁺CD25⁻ cells, anti-CD3 antibody and Mitomycin-C treated CD4⁻ cells, which are used as antigen presenting cells, were added. Well of CD4⁺CD25⁻ cells, anti-CD3 antibody and CD4⁻ cells were used as positive control. Well of CD4⁺CD25⁻ cells and CD4⁻ cells without anti CD3 antibody was used as negative control. Four days after the co-culture started, expression of CFSE on CD4⁺CD25⁻ cells in each well was detected by flow cytometer. n=3

BMT and induction of colitis. To assess the specific necessity for BM-derived IL-7 in the development and persistence of colitis, we performed an adoptive transfer experiment in combination with BMT using RAG-1^{-/-} and IL-7^{-/-} × RAG-1^{-/-} littermate recipients (Fig. 1A). Mice first were administered 10 mg/kg busulfan (Sigma-Aldrich) intraperitoneally, and thereafter they underwent whole-body irradiation with a total of 5 Gy to ablate their BM. On the next day, mice were reconstituted with 5×10^6 donor BM cells via a single tail vein injection and were used for adoptive transfer experiments after 4 wk recovery. For the adoptive transfer, CD4⁺ T cells were first isolated from spleens of C57BL/6 mice using the anti-CD4 (L3T4)-MACS system (Miltenyi Biotec, Auburn, CA) according to the manufacturer's instructions. Enriched CD4⁺ T cells (96–97% pure, as estimated by flow cytometry [FACS Calibur, Becton

Dickinson, Sunnyvale, CA) were then labeled with PE-conjugated anti-mouse CD4 (RM4-5; BD Pharmingen, San Diego, CA) and FITC-conjugated anti-CD45RB (16A; BD Pharmingen). CD4⁺CD45RB^{high} T cells were isolated using a FACS Aria cell sorter (Becton Dickinson). This population was > 98.0% pure on reanalysis. Mice were divided into four groups as follows: Group 1, IL-7^{-/-} × RAG-1^{-/-} mice (n = 8) that had been transplanted with male RAG-1^{-/-} BM cells and subsequently injected with CD4⁺CD45RB^{high} T cells; Group 2, IL-7^{-/-} × RAG-1^{-/-} mice (n = 8) that had been transplanted with IL-7^{-/-} × RAG-1^{-/-} BM cells and subsequently injected with CD4⁺CD45RB^{high} T cells; Group 3, RAG-1^{-/-} mice (n = 8) that had been transplanted with RAG-1^{-/-} BM cells and subsequently injected with 3 × 10⁵ CD4⁺CD45RB^{high} T cells; and Group 4, RAG-1^{-/-} mice (n = 8) that had been transplanted with IL-7^{-/-} × RAG-1^{-/-} BM cells and subsequently injected with CD4⁺CD45RB^{high} T cells.

All mice were observed for clinical signs such as hunched posture, piloerection, diarrhea, and blood in the stool. At autopsy, mice were assessed by a clinical score (3) that is the sum of three parameters: hunching and wasting, 0 or 1; colon thickening, 0–3 (0, no colon thickening; 1, mild thickening; 2, moderate thickening; 3, extensive thickening); and stool consistency, 0–3 (0, normal beaded stool; 1, soft stool; 2, diarrhea; 3, bloody stool) (4).

MSC transfer and time course detection of IL-7. IL-7^{+/+} MSC (1 × 10⁶) were transferred to IL-7^{-/-} × RAG-1^{-/-} mice via a single tail vein injection. One, 2, and 4 wk after the transfer, mice (n = 3) were sacrificed, and the expression of IL-7 mRNA in the BM, SP and colon was checked by RT-PCR, while IL-7 protein was checked by immunohistochemistry.

MSC transfer and induction of colitis. To assess the importance of MSC as the main source of IL-7 for the persistence of chronic colitis, we performed the following *in vivo* experiment. MSC were cultured and purified as described above. Mice were then injected with 1×10^6 IL-7^{+/+} or IL-7^{-/-} MSC via a single tail vein injection and were used for adoptive transfer experiments after 3 wk. Mice were divided into two groups as follows: Group 1, IL-7^{-/-} × RAG-1^{-/-} mice (n = 5) that had been transplanted with IL-7^{+/+} MSC and subsequently injected with CD4⁺CD45RB^{high} T cells; Group 2, IL-7^{-/-} × RAG-1^{-/-} mice (n = 5) that had been transplanted with IL-7^{+/+} MSC and subsequently injected with CD4⁺CD45RB^{high} T cells. All mice were observed for clinical signs and assessed for a clinical score as described above.

Simultaneous transfer of MSC and CD4⁺CD45RB^{high} T cells to RAG-2^{-/-} mice. We checked whether our cultured MSC could suppress colitis when they were transferred to RAG-2^{-/-} mice simultaneously with CD4⁺CD45RB^{high} T cells. Mice were divided into four groups as follows. Group 1: RAG-2^{-/-} mice were injected with 1×10^6 IL-7^{+/+} MSC via a single tail vein injection and simultaneously injected intraperitoneally with 3×10^5 CD4⁺CD45RB^{high} T cells (n = 3). Group 2: RAG-2^{-/-} mice were injected with 1×10^6 IL-7^{-/-} MSC via a single tail vein injection and simultaneously injected intraperitoneally with 3×10^5 CD4⁺CD45RB^{high} T cells (n = 3). Group 3: RAG-2^{-/-} mice were injected intraperitoneally with 3×10^5 CD4⁺CD45RB^{high} T cells alone (n = 3). Group 4: RAG-2^{-/-} mice were injected intraperitoneally with 3×10^5 CD4⁺CD45RB^{high} T cells and 3×10^5 CD4⁺CD25⁺ T cells (n = 3). All mice were observed for clinical signs and were assessed for a clinical score as described above.

Adoptive transfer of different numbers of IL-7^{+/+} MSC in CD4⁺CD45RB^{high} T cells transfer. 1x10⁶ (Gr. 1), 1x10⁵ (Gr. 2) or 1x10⁴ (Gr. 3) IL-7^{+/+} MSC were transferred to IL-7^{-/-} x RAG-1^{-/-} mice via a single tail vein injection. Four weeks after MSC transfer, each mouse was transferred with 3 x 10⁵ CD4⁺CD45RB^{high} T cells sorted from splenocyte of C57BL/6 mice intraperitoneally. Fourteen weeks after MSC transfer, mice were sacrificed and analyzed. n=5 in each group.

Multiple injection of MSC in CD4⁺CD45RB^{high} T cells transfer colitis. 3 x 10⁵ CD4⁺CD45RB^{high} T cells were transferred into RAG-2^{-/-} mice intraperitoneally. At the same time, 1 x 10⁶ IL-7^{+/+} MSC (IL-7^{+/+} MSC) and 1 x 10⁶ IL-7^{-/-} MSC (IL-7^{+/+} MSC) were transferred intravenously. In addition, they were also transferred 1 and 2 weeks after the RB^{high} T cells injection. As positive control, CD4⁺CD45RB^{high} T cells alone were transferred into RAG-2^{-/-} mice (RB^{high}). As negative control, 3 x 10⁵ CD4⁺CD25⁺ regulatory T cells were co-transferred with CD4⁺CD45RB^{high} T cells into RAG-2^{-/-} mice (RB^{high} + Treg). n=5 in each group. Six weeks after MSC transfer mice were sacrificed and analyzed.

MSC differentiation assay. IL-7^{+/+} MSC and IL-7^{-/-} MSC were induced to differentiate to adipocytes, osteocytes and chondrocytes using the Mouse Mesenchymal Stem Cell Functional Identification Kit® (R&D Systems). After differentiation, adherent cells were stained with anti-FABP-4 antibody for adipocytes, anti-osteopontin antibody for osteocytes and anti-collagen-II antibody for chondrocytes, together with polyclonal anti-IL-7 antibody in multicolor immunohistochemistry. Differentiated cells were also collected using trypsin-EDTA, RNA was purified and RT-PCR was performed to check the expression of IL-7 mRNA.

Histological examination. Tissue samples were fixed in PBS containing 6% neutral-buffered formalin. Paraffin-embedded sections (5 mm) were stained with H&E. Three tissue samples from the proximal, middle, and distal parts of the colon were prepared. The sections were analyzed without prior knowledge of the type of T cell reconstitution or treatment. The area most affected was graded by the number and severity of lesions. The mean degree of inflammation in the colon was calculated using a modification of a previously described scoring system (4), as follows. Mucosal damage: 0, normal; 1, 3–10 intraepithelial leukocytes (IEL)/high power field (HPF) and focal damage; 2, >10 IEL/HPF and rare crypt abscesses; 3, >10 IEL/HPF, multiple crypt abscesses and erosion/ulceration. Submucosal damage: 0, normal or widely scattered leukocytes; 1, focal aggregates of leukocytes; 2, diffuse leukocyte infiltration with expansion of submucosa; 3, diffuse leukocyte infiltration. Muscularis damage: 0, normal or widely scattered leukocytes; 1, widely scattered leukocyte aggregates between muscle layers; 2, leukocyte infiltration with focal effacement of the muscularis; 3, extensive leukocyte infiltration with transmural effacement of the muscularis.

Tissue preparation. Single cell suspensions were prepared from SP, MLN, LP, and BM as previously described (2). To isolate LP CD4⁺ T cells, the entire length of colon was opened longitudinally, washed with PBS, and cut into small pieces. The dissected mucosa was incubated with Ca²⁺-, Mg²⁺-free Hanks' balanced salts solution containing 1 mM DTT (Sigma-Aldrich) for 45 min to remove mucus and then treated with 3.0 mg/ml collagenase (Roche Diagnostics GmbH, Germany) and 0.01% DNase (Worthington Biomedical Co., Freehold, NJ) for 2 h. The cells were pelleted twice through a 40% isotonic

Percoll solution and then subjected to Ficoll–Hypaque density gradient centrifugation (40%/75%). Enriched LP CD4⁺ T cells were obtained by positive selection using anti-CD4 (L3T4) MACS magnetic beads. The resultant cells analyzed by flow cytometry contained > 95% CD4⁺ cells. BM cells were obtained by flushing two femurs with cold RPMI-1640.

RT–PCR. Total RNA was isolated by using Nucleospin® (MACHERY-NAGEL). Aliquots of 0.5 mg of total RNA were used for complementary DNA synthesis in 20 mL of reaction volume by using QuantiTect Reverse Transcription Kit® (QIAGEN). One microliter of reverse transcription product was amplified with 0.25 U of rTaq DNA polymerase (Toyobo) in a 50 μ l reaction. Sense and antisense primers and the cycle numbers for the amplification of each gene were as follows: IL-7, forward 5'–GCCTGTCACATCATCTGAGTGCC–3' and reverse 5'–CAGGAGGCATCCAGGAACTTCTG–3' (35 cycles); glyceraldehyde-3-phosphate dehydrogenase (G3PDH) forward 5'–TGAAGGTCGGTGTGAACGGATTTGGC–3' and reverse 5'–CATGTAGGCCATGAGGTCCACCAC–3' (30 cycles). The amplification for each gene was logarithmic under these conditions. PCR products were separated on 1.8% agarose gels, stained with ethidium bromide, and visualized with a Lumi-Imager F1 (Roche Diagnostics).

Real time PCR. Total RNA was isolated by using Nucleospin® (MACHERY-NAGEL). Aliquots of 0.5 mg of total RNA were used for complementary DNA synthesis in 20 mL of reaction volume by using QuantiTect Reverse Transcription Kit® (QIAGEN). To validate gene expression changes, quantitative RT-PCR analysis was performed by Applied Biosystems 7500 using validated TaqMan Gene Expression Assays (Applied Biosystems). The

TaqMan probes and primers for mouse IL-7 (assay identification number Mm01295805_m1) was Assay-on-Demand gene expression products (Applied Biosystems). The mouse b-actin gene was used as endogenous control (catalog number 4352933E; Applied Biosystems). The thermal cycler conditions were as follows: hold for 10min at 95°C, followed a cycle of 95°C for 15s and 60°C for 1min for 50 cycles. Amplification data were analyzed with an Applied Biosystems Sequence Detection Software version 1.3, and the relative mRNA amounts and range were determined by comparative CT method. Briefly, we normalized each set of samples using the difference in threshold cycles (CT) between the sample gene and housekeeping gene (b-actin): $\Delta CT = (CT_{\text{sample}} - CT_{\text{b-actin}})$. Relative mRNA levels were calculated by the expression $2^{-\Delta\Delta CT}$ where $\Delta\Delta CT = \Delta CT_{\text{sample}} - \Delta CT_{\text{standard}}$. We used IL-7^{+/+} BMC as standard in figure 2A. We used IL-7^{+/+} pre MSC as standard in figure 2D. We used donor IL-7^{+/+} MSC as standard in supplemental figure 5H.

Immunohistochemistry. Consecutive colon cryostat sections (5 mm) were fixed and stained with anti-CD4 mAb (BD Pharmingen) or biotin-conjugated polyclonal anti-IL-7 antibody (R&D Systems). Alexa Fluor® 584 goat anti-rat IgG or Alexa Fluor® 488 streptavidin (Molecular Probes) was used as secondary reagent. All microscopy was carried out on a BioZERO BZ8000 (Keyence, Tokyo).

Cytokine ELISA. To measure cytokine production, 1×10^5 LP CD4⁺ T cells were cultured in 200 μ l culture medium at 37 °C in a humidified atmosphere containing 5% CO₂ in 96-well plates (Costar, Cambridge, MA) precoated overnight at 4 °C with 5 mg/ml hamster anti-mouse CD3e mAb (145-2C11, BD Pharmingen) and hamster 2 mg/ml anti-mouse CD28 mAb (37.51, BD

Pharmingen) in PBS. Culture supernatants were removed after 48 h and assayed for cytokine production. Cytokine concentrations were assayed by specific ELISA according to the manufacturer's recommendation (R&D Systems).

Flow cytometry. To detect the surface expression of a variety of molecules, isolated splenocytes, BM, or LP mononuclear cells were preincubated with an Fc γ R-blocking mAb (CD16/32; 2.4G2, BD Pharmingen) for 20 min followed by incubation with specific FITC-, PE-, PerCP-, APC- or biotin-labeled antibodies for 30 min on ice. FITC- or PE or APC-conjugated Sca1 (D7), Biotin-conjugated anti-mouse IL-7R α (A7R34), APC-conjugated anti-mouse PDGFR α (APA5), APC-conjugated anti-mouse PDGFR β (APB5), PE-conjugated anti-mouse CD105 (MJ7/18), PE-conjugated anti-mouse CD146 (P1H12), PE-conjugated anti-mouse FLK1 (Avas12a1) and biotin-conjugated anti-mouse VEGFR3 (AFL4) were obtained from eBioscience (San Diego, CA). The following mAbs were obtained from BD Pharmingen: FITC- or PerCP-conjugated anti-mouse CD3 (RM4–5), FITC-, PE-, PerCP- or APC-conjugated anti-mouse CD4 (16A), PE-conjugated CD11b (M1/70), FITC-conjugated Gr-1 (RB6-8C5), FITC-conjugated TER119 (TER119), FITC-conjugated CD45 (30-F11), FITC-conjugated CD34 (RAM34), FITC- or APC-conjugated anti-mouse CD45RB (IM7), PE-conjugated anti-mouse CD44 (PC61), FITC-conjugated anti-mouse CD62L (H1.2F3), FITC-conjugated anti-mouse CD69 (A20), PE-conjugated anti-mouse CD73 (TY23). Biotinylated antibodies were detected with PE–streptavidin (BD Pharmingen). Standard two-, three-, or four-color flow cytometric analyses were performed using a FACS Calibur flow cytometer and

CellQuest software. Background fluorescence was assessed by staining with control irrelevant isotype-matched mAbs.

Statistical analysis. We examined the normality of the distribution of results in each group. If results from either group were not normally distributed, we assessed the difference between any two groups using the Mann–Whitney *U*-test. If results from both groups were normally distributed, we assessed the variance of the population to which each group belonged using the *F*-test. When the populations were homoscedastic, we assessed the difference between two groups using Student's *t*-test. In the absence of homoscedasticity, we assessed the difference using Welch's *t*-test. We used Statcell software for all statistical analyses. Results are expressed as the mean \pm SEM. Differences were considered significant when $P < 0.05$.

Supplemental Figure Legends

Supplemental Figure 1

Expression of IL-7R α and CD69 on LP CD4⁺ T cells by FACS.

BM, SP, MLN, and LP CD3⁺CD4⁺ T cells from IL-7^{+/+} \rightarrow IL-7^{-/-}, IL-7^{+/+} \rightarrow IL-7^{-/-}, IL-7^{+/+} \rightarrow IL-7^{+/+} or IL-7^{-/-} \rightarrow IL-7^{+/+} mice were isolated at 6 wk after transfer, and the expression of IL-7R α and CD69 was determined by flow cytometry.

Supplemental Figure 2

Phenotypic characterization of cultured MSC and freshly isolated bone marrow cells from IL-7^{+/+} x RAG-1^{-/-} or IL-7^{-/-} x RAG-1^{-/-} mice

Hematopoietic markers, CD11b, Gr-1, TER119, CD3, CD45, CD34, endothelial markers, CD146, CD309, VEGFR and MSC markers, Sca-1, CD140a, CD140b, CD73, CD105 on cultured MSC and freshly isolated bone marrow cells from IL-7^{+/+} x RAG-1^{-/-} or IL-7^{-/-} x RAG-1^{-/-} mice were checked by flow cytometry. MSC were used after more than 7th passage. Representatives of 5 separate samples in each group.

Supplemental Figure 3

Both IL-7^{+/+} MSC and IL-7^{-/-} MSC suppressed proliferation of activated CD4⁺ T cells *in vitro*

(A) Experimental design. First, IL-7^{+/+} MSC (Gr. 1) and IL-7^{-/-} MSC (Gr. 1) were cultured in respective well. The next day, CFSE-labeled CD4⁺CD25⁻ cells, anti-CD3 antibody and Mitomycin-C treated CD4⁻ cells, which are used as antigen presenting cells, were added. Well of CD4⁺CD25⁻ cells, anti-CD3 antibody and CD4⁻ cells were used as positive control (Gr. 3). Well of CD4⁺CD25⁻ cells and CD4⁻ cells without anti CD3 antibody was used as negative control (Gr. 4). Four days after the co-culture started, expression of CFSE on CD4⁺CD25⁻ cells in each well was detected by flow cytometer. n=3 **(B)** Expression of CFSE on collected CD4⁺CD25⁻ cells in each well. Representative results of three separate samples in each group. **(C)** The number of collected CD4⁺CD3⁺ cells in each well. Data are shown as the mean \pm SEM. N.S., not significant. * $P < 0.01$. **(D)** Percentage of divided cells was calculated by the result of CFSE expression. Data are shown as the mean \pm SEM. N.S., not significant. * $P < 0.01$.

Supplemental Figure 4

Phenotype of CD4⁺ T cells isolated from BM, SP, and LP of IL-7^{+/+} MSC mice and IL-7^{-/-} MSC mice 7 wk after transfer of CD4⁺CD45RB^{high} T cells

Expression of IL-7R α , CD69, CD44 and CD62L on the CD3⁺CD4⁺ gated cells in BM, SP, and LP was checked by flow cytometry. Representative results of five separate samples in each group.

Supplemental Figure 5

Different numbers of IL-7^{+/+} MSC were transferred into IL-7^{-/-} x RAG-1^{-/-} mice 4 weeks before CD4⁺CD45RB^{high} T cells transfer.

(A) 1x10⁶ (Gr. 1), 1x10⁵ (Gr. 2) or 1x10⁴ (Gr. 3) IL-7^{+/+} MSC were transferred to IL-7^{-/-} x RAG-1^{-/-} mice. Four weeks after the transfer, each mouse was transferred with CD4⁺CD45RB^{high} T cells. Ten weeks after, mice were sacrificed and analyzed. n=5 in each group. (B) Gross appearance of the colon, MLN and SP from mice of each group at 14 wk after MSC administration. (C) Clinical scores determined at 14 wk after MSC administration as described in Materials and Methods. Data are shown as the mean \pm SEM. N.S., not significant. * $P < 0.01$. (D) Histopathology of colon of the indicated mice 14 wk after MSC transfer. Original magnification $\times 200$. (E) Histological scores. Data are shown as the mean \pm SEM. N.S., not significant. * $P < 0.05$. (F) Absolute cell number of LP CD3⁺CD4⁺ T cells from colon 14 wk after MSC transfer. Data are shown as the mean \pm SEM. N.S., not significant. * $P < 0.01$. (G) Cytokine production by LP CD4⁺ T cells. LP CD4⁺ T cells were isolated 14 wk after MSC transfer and stimulated *in vitro*. IFN- γ , TNF- α , and IL-17 concentrations in culture supernatants were measured by ELISA. Data are shown as the mean \pm SEM *

N.S., not significant. $P < 0.05$. **(H)** Relative expression of IL-7 mRNA in the BM or colon from the indicated mice was determined by Real time-PCR. Data are shown as the mean \pm SEM *

Supplemental Figure 6

Multiple injection of MSC could not suppressed CD4⁺CD45RB^{high} T cells transfer colitis

(A) CD4⁺CD45RB^{high} T cells were transferred into RAG-2^{-/-} mice. At the same time, IL-7^{+/+} MSC (IL-7^{+/+} MSC) and IL-7^{-/-} MSC (IL-7^{+/+} MSC) were transferred intravenously. In addition, they were also transferred 1 and 2 weeks after the RB^{high} T cells injection. As positive control, CD4⁺CD45RB^{high} T cells alone were transferred into RAG-2^{-/-} mice (RB^{high}). As negative control, CD4⁺CD25⁺ regulatory T cells were co-transferred with CD4⁺CD45RB^{high} T cells into RAG-2^{-/-} mice (RB^{high} + Treg). n=5 in each group. **(B)** Gross appearance of the colon, MLN and SP from mice of each group at 6 wk after MSC administration. **(C)** Clinical scores determined at 14 wk after MSC administration as described in Materials and Methods. Data are shown as the mean \pm SEM. N.S., not significant. * $P < 0.01$. **(D)** Histological scores. Data are shown as the mean \pm SEM. N.S., not significant. * $P < 0.05$. **(E)** Histopathology of colon of the indicated mice 6 wk after MSC transfer. Original magnification $\times 200$. **(F)** Absolute cell number of LP CD3⁺CD4⁺ T cells from colon 6 wk after MSC transfer. Data are shown as the mean \pm SEM. N.S., not significant. * $P < 0.01$. **(G)** Cytokine production by LP CD4⁺ T cells. LP CD4⁺ T cells were isolated 6 wk after MSC transfer and stimulated *in vitro*. IFN- γ , TNF- α , and IL-17

concentrations in culture supernatants were measured by ELISA. Data are shown as the mean \pm SEM * N.S., not significant. $P < 0.05$.

Supplemental Reference

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