Poly(I:c)-priming Improved the Therapeutic Efficacy of Mesenchymal Stromal Cells on Experimental Colitis by Promoting the Expression of Indoleamine 2,3-dioxygenase

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Research

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Abstract

Background: Inflammatory bowel disease is a chronic and excessive inflammation of the colon and small intestine. We previously reported that priming of mesenchymal stromal cells (MSC) with poly(I:C) induced them to express indoleamine 2,3-dioxygenase (IDO). We tried to find out whether the poly(I:C)-primed MSCs have better therapeutic efficacy on the experimental colitis in the IDO1-dependent manner.

Methods: To compare the therapeutic effects between the naïve MSCs and primed MSCs on murine colitis, mice (C57BL6) were administered with 2.5% dextran sodium sulfatein drinking water for 5 days and injected with MSCs intraperitoneally on days 1 and 3 following DSS ingestion. The disease activity index score and body weight loss were significantly improved after injection of the primed MSCs.

Results: Mice receiving the poly(I:C)-primed MSCs showed a reduced disease activity index and less weight loss. Colon tissue from the same mice presented attenuated pathological damage, increased Paneth cells, increased IDO1-expressing cells, and better proliferation of enterocytes. The primed MSC treatment upregulated the mRNA expression of intestinal stem cell markers (Lgr5, Olfm4, and Bmi1), enterocyte differentiation markers (Muc2, Alpi, Chga, and occludin), and regulatory T (Treg) cells (Foxp3). The same treatment decreased inflammatory cell infiltration to lymphoid organs and the level of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, and MCP-1) in colon tissue. Notably, in vivo pharmacologic inhibition of the IDO1 activity blocked the Foxp3 up-regulation in colon tissue and diminished the protective effects of the primed MSC.

Conclusions: The priming of MSCs with poly(I:C) is a promising new strategy to improve the therapeutic efficacy of MSC and is worth further research.

Background

Mesenchymal stromal cells (MSC) have been tried to treat various inflammatory or auto-immune disorders since they are immune-modulatory, are not as immunogenic as other allogeneic cell types, and can contribute to tissue repair (1, 2). They are easily isolated and expanded from bone marrow (BM) and even medical waste, such as adipose and umbilical tissues (3). Their therapeutic efficacy on inflammatory bowel disease (IBD) was suggested by previous preclinical and clinical studies (4). MSC injections improved the murine experimental colitis by down-regulating Th1, Th17 responses (5, 6), up-regulating Treg responses (7, 8), and inducing M2 macrophages (9). MSCs secrete several soluble factors, including transforming growth factor-β (TGF-β), prostaglandin E2 (PGE2), nitric oxide (NO), and indoleamine 2,3-dioxygenase (IDO) to suppress activated T cells (10–12). Secretion of these factors can be up-regulated by pro-inflammatory cytokines, such as interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-1β (13). Cell surface molecules, including programmed cell death (PD)-L1 and Fas ligand (FasL), are also induced by IFN-γ treatment and mediate the T-cell suppression via cell-contact-dependent mechanisms (14, 15).
Recently, the allogeneic adipose-derived MSC product (Cx601) showed favorable outcomes in the phase III clinical trial and obtained the landmark approval in Europe for the treatment of complex perianal fistulas in Crohn's disease (16). Other studies also found the positive effects of locally injected MSCs, but they indicated that the efficacy decreased over time and that there was perhaps a need for repeated treatments (17). In contrast to the success of local injection, systemic MSC therapy given intravenously for the luminal IBD has shown limited efficacy and inconsistent results (18). More mechanistic studies in representative murine models of IBD are needed to bring the immune modulation by MSC to the clinic.

In our previous work, we primed murine BM-derived MSCs with various toll-like receptor (TLR) ligands and found that only the TLR3 ligand polyinosinic-polycytidylic acid [poly(I:C)] significantly increased the expression of IDO. Furthermore, poly(I:C)-treated MSCs could improve the pathologic scores of Dextran sulfate sodium (DSS)-induced colitis more effectively than could unstimulated MSCs (19). In the present study, we demonstrated that the poly(I:C)-primed MSCs increased the Treg frequency, decreased inflammation, stimulated epithelial regeneration, and had better therapeutic efficacy on the murine IBD model than did unstimulated MSCs.

Materials And Methods

Mice

Female C57BL/6 mice (9 ~ 10 weeks old) were purchased from Japan SLC, Inc. (Shizuoka, Japan). All animal experiments have been approved by the Institutional Animal Care and Use Committees of the Catholic University of Korea (Seoul, Korea).

Priming of murine BM-derived MSCs

The BM-derived MSCs were isolated from C57BL/6 mice and expanded, as described in our previous work (19). In brief, BM cells were flushed out from femurs and tibias, plated in 75 cm² tissue culture flasks at a concentration of $1 \times 10^6$ cells/mL in the complete culture medium, and incubated at 37°C and 5% CO₂. Non-adherent cells were removed after three days, and the remaining cells were passaged into a new flask when the cells reached 70 ~ 80% confluency. To do priming, we harvested cells at the 90% confluency and plated them in 12-well plates ($5 \times 10^4$ cells/ well) in the complete culture medium supplemented with recombinant mouse IFN-γ (100 ng/mL, R&D Systems, Minneapolis, MN, USA). Poly(I:C) (TLR3 ligand, 10 µg/mL, Sigma-Aldrich, St Louis, MO, USA) was added to the culture medium for stimulation. The primed MSCs were collected after 24 h and used for in vitro and in vivo experiments.

DSS-induced murine colitis and MSC treatments

Experimental colitis was induced by administration of 2.5% DSS (molecular weight 36,000 ~ 50,000; MP Biomedicals, Santa Ana, CA, USA) in drinking water ad libitum for six days (day 0 ~ 5). Nine-week-old mice were randomly allocated into one of three groups: DSS only (DSS control), DSS with unstimulated MSCs treatment (DSS + MSCs), and DSS with the treatment of MSCs stimulated with IFN-γ and poly(I:C)
(DSS + primed-MSCs). Unstimulated MSCs or the primed (stimulated) MSCs were injected intraperitoneally (i.p.) on days 1 and 3. We assessed the severity of colitis using body weight and the disease activity index (DAI), which evaluates stool consistency and the presence of fecal blood as previously described (20). All mice were sacrificed on day 9 to harvest the spleen, mesenteric lymph node (mLN), and colon tissue.

**L-1MT preparations**

To prepare L-1-methyl tryptophan (L-1MT, Sigma-Aldrich, St. Louis, MO) for oral gavage, 1 g of L-1MT was added to a 15-ml conical tube with 7.8 ml Methocel/Tween [0.5% Tween 80/ 0.5% Methylcellulose (v/v in water; both from Sigma-Aldrich)]. The following day, the L-1MT concentration was adjusted to 85 mg/ml by adding an additional 4 ml Methocel/Tween and mixing again. For *in vitro* use, 1-MT was prepared as a 100 mmol/L stock in 0.1 N NaOH, adjusted to pH 7.4 and stored at -20 °C protected from light.

**Hematoxylin-eosin (H&E) staining and Immunohistochemistry (IHC)**

We subjected Formalin-fixed, paraffin-embedded tissue sections to H&E staining for microscopic examination. Slides were scored by a pathologist (blinded to experimental group). Pathologic severity of IBD was assessed by three parameters (epithelial loss, crypt destruction, and inflammatory cell infiltration), as described before (20).

Tissue sections (4 µ) were mounted on super frost glass sliders and deparaffinized in xylene and a graded series of ethanol, followed by antigen retrieval. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide. Nonspecific binding sites were saturated by exposure to 10% normal goat serum diluted in phosphate buffered saline (PBS) for 60 min. We incubated slides overnight at 4°C with primary antibodies against mouse Ki-67 (1:100 dilution, Abcam, CB, UK), Lysozyme (1:250 dilution, Abcam), IDO-1 (1: 400 dilution, Biolegend, San Diego, CA), then washed with PBS for 10 min. Biotinylated goat anti-rabbit IgG and rabbit anti-rat IgG (Vector Laboratories, Burlingame, CA) secondary antibodies were applied to tissue sections, and the slides were incubated at room temperature for 30 min. After the sections were washed and incubated for 30 minutes with peroxidase-conjugated streptavidin (Dako, Glostrup, Denmark) at room temperature, 3,3’-diaminobenzidine was added to visualize antigens. Sections were counterstained with Mayer’s hematoxylin, dehydrated, cleared, and mounted. We prepared negative control tissue samples in the same manner as described above, except that the primary antibody was omitted or replaced with an isotype control antibody (R&D Systems, Minneapolis, MN).

**Quantitative RT-PCR**

We isolated total RNA from colon homogenates with Trizol® (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. One microgram of total RNA was reverse transcribed into cDNA. We did quantitative assessment of target mRNA levels by quantitative RT-PCR using a CFX96TM real-time PCR detection system (Bio-Rad, Hercules, CA, USA). The quantity of mRNA was calculated using the $2^{-\Delta\Delta Ct}$
method, and the level of β-actin was used to normalize total RNA quantities. The sequences of forward and reverse primers are shown in Table 1.
Table 1  
Primers used for qPCR amplification.

| Gene    | Forward sequence (5′-3′) | Reverse sequence (5′-3′) |
|---------|--------------------------|--------------------------|
| Lgr5    | ACCCGCCAGTCTCTCATC       | GCATCTAGGCAGCAAGGATGG    |
| OLFM4   | TGGCCCTTGGAAGCTGTAGT     | ACCTCCTTGCCATAGCGAA      |
| Bmi1    | GCCACTACCATAATAGAATGTCT  | TTGTGAACCTGGACAATCACAAA  |
| Axin2   | GCAAACTTTTCGCCAACCGTG    | CTCTGGAGCTTTCTAATCTGGCC  |
| Muc2    | GCTGACGAGTGGTTGTGAATG    | GATGAGGTGGCAGACAGGAGAC   |
| Alpi    | GGCTACACACTTACAGG        | AGCTTCCGGTGAATATTGGGA    |
| Chga    | AGGTGATGAAATGCCTTCG      | GGTGTCGCAAGTAGAGAGGA     |
| Occludin| GGACCCTGACCACCTATGAAACAGACTAC | ATAGGTGGATATTCCCTGACCCAGTC |
| IL-1β   | GCAACTGTTTCTGAACTCACT    | ATCTTTTGTTGGTCCGTCAACT   |
| TNF-α   | GGAACACGTCGTGGGATAATG    | GGCAGACTTTGGGATGCTTTTT   |
| IL-6    | TCCATCCAGTTGCCCTTCCTG    | GGTCTGTTGGGAGTGTTATC     |
| MCP-1   | CTCACCTGCTGCTACTCATTC    | GCTTGAGGTGGTTGGTGAAAGAA  |
| Foxp3   | ACAACCTGAGCCTGACAAGTT    | GCCCACCCTTTCTTGTTTTTG    |
| IL-10   | AGGGCCCTTTGCTATGGGT      | TGGCCACAGTTTTCAAGGAT     |
| IDO-1   | ATTTGGTGGAATCAGGCAGCTCC | ACAAAAGTCACGCATCCATCTTAAA |

Lgr5, Leucine Rich Repeat Containing G Protein-Coupled Receptor 5; OLFM4, Olfactomedin 4; Bmi1, B cell-specific Moloney murine leukemia virus integration site 1; Axin2, Axis inhibition protein 2; Muc2, Mucin 2; Alpi, Alkaline phosphatase, intestinal; Chga, Chromogranin A; IL-1β, Interleukin 1 beta; TNF-α, Tumor necrosis factor alpha; IL-6, Interleukin 6; MCP-1, Monocyte chemoattractant protein 1; Foxp3, Forkhead box P3; IL-10, Interleukin 10; IDO-1, Indoleamine 2,3-dioxygenase; COX2, Cyclooxygenase-2; PTEGS3, Prostaglandin E synthase 3
| Gene      | Forward sequence (5′-3′) | Reverse sequence (5′-3′) |
|-----------|--------------------------|--------------------------|
| COX2      | CCAGCACCCTACCCAT CAGTT  | ACCCAGGTCCCTCGCT TATGA   |
| PTEGS3    | ATCACATGGGTTGCTG ATGAGGA| AGGCGATGACAAACAG CCCTTAC|
| β-actin   | AGCTCGCGTTTACAC CTTT    | AAGCCATGCCAAGTT GTCT     |

Lgr5, Leucine Rich Repeat Containing G Protein-Coupled Receptor 5; OLFM4, Olfactomedin 4; Bmi1, B cell-specific Moloney murine leukemia virus integration site 1; Axin2, Axis inhibition protein 2; Muc2, Mucin 2; Alpi, Alkaline phosphatase, intestinal; Chga, Chromogranin A; IL-1β, Interleukin 1 beta; TNF-α, Tumor necrosis factor alpha; IL-6, Interleukin 6; MCP-1, Monocyte chemoattractant protein 1; Foxp3, Forkhead box P3; IL-10, Interleukin 10; IDO-1, Indoleamine 2,3-dioxygenase; COX2, Cyclooxygenase-2; PTEGS3, Prostaglandin E synthase 3

**Flow cytometric analysis**

Single-cell suspensions were stained in FACS buffer at 4 °C for 30 min. We analyzed Samples using an LSRII (BD Pharmingen, San Diego, CA). The following antibodies against mouse antigens were purchased from BD Pharmingen (San Diego, CA): BV605-conjugated anti-CD11b, FITC-conjugated anti-CD11c, BV450-conjugated anti-CD4, FITC-conjugated CD25, and PE-conjugated anti-Foxp3.

**Treg generation**

We did the following experiments to analyze the effect of MSCs on T-cell proliferation. We isolated untouched T cells from splenocytes using the Pan T Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany). We cocultured $2 \times 10^5$ splenocytes with or without MSCs (primed or unstimulated) in the presence or absence of 2 l/ml anti-CD3/CD28 (ebioscience, San Diego, CA, USA) plus 10 ng/ml recombinant murine TGF-β (R&D Systems) and 50 ng/ml recombinant murine IL-2 (R&D Systems) for 72 hours. Then, T cells were harvested and surface stained for CD4, CD25, and Foxp3. A competitive IDO1 inhibitor, L-1-MT (Sigma-Aldrich, St. Louis, MO, USA) was added to some wells.

**Statistical analysis**

All values are expressed as mean ± standard error of the mean. We did statistical comparisons between groups using a parametric independent sample t-test if there were ≥ 5 animals per group, or used the Mann-Whitney test if there were < 5 animals per group.

**Results**

The poly(I:C)-primed MSCs were more effective than unstimulated MSCs to ameliorate the DSS-induced colitis in mice.

To identify the immunomodulatory effects of the poly(I:C)-primed MSC, we used the murine DSS-induced colitis model (Fig. 1A). MSCs were primed with poly(I:C) as described before (26) and injected i.p. to mice...
on days 1 and 3 (DSS + primed MSCs group). Other mice were treated with the same volume of saline (DSS group) or unstimulated MSC (DSS + MSCs group) on days 1 and 3. Compared to the DSS group and DSS + unstimulated MSC group, the DSS + primed MSCs group had reduced DAI scores and less weight loss (Fig. 1B-C). Additionally, the primed MSC treatment shortened the colon length less, which is the anatomic marker of colonic inflammation (Fig. 1D). A pathologic examination confirmed the symptomatic improvement of the DSS-induced colitis, which was caused by the primed MSC. Figure 1E shows that the typical pathologic findings of the DSS-induced colitis (epithelial loss, crypt destruction, and inflammatory cell infiltration) were significantly ameliorated by the unstimulated MSC and further by the primed MSC. These results demonstrated the improved anti-inflammatory effects of the primed MSC compared to the unstimulated MSC.

The poly(I:C)-primed MSCs significantly stimulated the intestinal stem cell (ISC) proliferation, enterocyte differentiation, and epithelial regeneration

We did the IHC staining of a cell proliferation marker, Ki-67, using colon tissues, which were harvested on day 9. The Ki-67 expression was prominently increased in the DSS + primed MSC group more than in other groups, suggesting the intestinal epithelial layer was recovered significantly faster (Fig. 2A). Next, we investigated the ISC proliferation and Wnt/β-catenin signaling pathway, since they play a pivotal role in maintaining intestinal homeostasis (21). Total RNA was isolated from colon tissues, and the mRNA of target genes was quantified by a real-time RT-PCR method. Markers of active ISC (Lgr5 and OLFM4) and quiescent ‘4+’ ISC (Bmi1) were significantly increased in the primed MSC group more than in the other groups. The expression of Axin2, a target gene of the Wnt/β-catenin signaling pathway, was increased in the primed MSC group (Fig. 2B). The mRNA levels of Muc2 (goblet cells), Alpi (enterocytes), Chga (enteroendocrine cells), and occludin (epithelial tight junction) were prominently upregulated in the primed MSC group more than in the other groups. (Fig. 2C). The IHC staining of lysozyme revealed that the primed MSC treatment significantly increased Paneth cells and preserved crypt structures better than the unstimulated MSC did (Fig. 2D). These results confirmed that the poly(I:C) priming, i.e., TLR3 stimulation, increased the MSC’s efficacy in promoting intestinal epithelial regeneration and maintain homeostasis.

**The poly(I:C)-primed MSC decreased inflammatory response in the spleen, mLN, and colon tissue**

IBD is a chronic inflammatory state of the gastrointestinal tract, which involves effector T cells and inflammatory cytokines (1). Therefore, we studied the anti-inflammatory and immunomodulatory effects of the primed MSC. Spleen, mLN, and colon tissues were harvested on day 9, as described in Fig. 1A. Like other findings, the primed MSC treatment reduced the inflammatory cell infiltration into lymphoid organs more effectively than the unstimulated MSC did. The numbers of monocytes (CD11b+), dendritic cells (CD11c+), and CD4+ T cells significantly decreased in the spleen and mLN from mice that had received the primed MSC treatment (Fig. 3A-C). The mRNA levels of inflammatory cytokines (IL-1β, TNF-α, and IL-
6) and a chemotactic factor (MCP-1) in colon tissue decreased more in the primed MSC group than in other groups (Fig. 3D). The mRNA expression of immune-suppressive cytokine (IL-10) in colon tissue was elevated in the primed MSC group (Fig. 3F). These findings can account for the increased efficacy of the poly(I:C)-primed MSC on the IBD.

The poly(I:C)-primed MSC expanded Treg cells in an IDO1-dependent manner

The Foxp3+ Treg cells were functionally defective or its frequency was significantly lower in active IBD patients (22). Interestingly, the antiTNFα therapy, such as infliximab, significantly increased the frequency of functional Foxp3+ Treg cells in patients with active IBD (23). In this study, we investigated the change of Foxp3+ Treg cell frequencies in lymphoid organs and colon tissue after treatments of the poly(I:C)-primed or unstimulated MSCs for DSS-induced colitis. The proportion of Treg (CD25+Foxp3+CD4+) to CD4+ T cells in spleen was significantly higher in the primed MSC group than in the unstimulated MSC group and the DSS control group. Difference of Treg proportion between the primed and unstimulated MSC treatments was not prominent in mLN (Fig. 4A). The mRNA of Foxp3 in colon tissue increased considerably more in the primed MSC group than in the other groups. The unstimulated MSC could not increase the Foxp3 expression compared to the DSS control group, although they showed some anti-inflammatory effects on the IBD. We suppose that the ability of MSCs to induce the Treg cell expansion is the most important improvements induced by the poly(I:C) priming. To further confirm the capacity of the primed MSCs for expanding Treg, we did in vitro experiments. In brief, T cells were isolated from splenocytes of B6 mice and co-cultured with the primed or unstimulated MSC. The primed MSC caused a significantly higher increase of Treg (CD25+Foxp3+) proportion to CD4+ T cells than the unstimulated MSCs did (Fig. 4C). Since we demonstrated that the poly(I:C) priming of MSC induced the considerable IDO1 up-regulation (19), we tried to find out whether the primed MSC promoted Treg cells in an IDO1-dependent manner. Figure 4D shows that a competitive IDO1 inhibitor, L-1-MT, diminished the increment of Treg (CD25+Foxp3+) proportion induced by the primed MSCs, confirming our hypothesis.

The poly(I:C)-primed MSCs increased the expression of colonic IDO1 and COX2

We explored how the IDO1 and PGE2 pathways were affected by the MSC treatment, since they promote intestinal homeostasis by limiting inflammatory responses and protecting the epithelium (24). We did the IHC staining of IDO1 using colon tissues, which were harvested on day 9, as described in Fig. 1A. The primed MSC treatment induced a significant increment of IDO1-expressing cells in intestinal crypts than the unstimulated MSCs did, suggesting the role of IDO1 in maintaining the intestinal epithelial layer (Fig. 5A). The real-time RT PCR confirmed the same finding for the IDO1 expression (Fig. 5B). We found that the poly(I:C)-primed MSCs induced a significantly higher expression of Cyclooxygenase 2 (COX2)
and Prostaglandin E synthase 3 (PTGES3) genes in colon tissue than did the unstimulated MSC, suggesting that the local PGE2 level may be increased in the inflamed site (Fig. 5B).

Pharmacologic IDO1 inhibition decreased the therapeutic efficacy of the poly(I:C)-primed MSCs on the DSS-induced colitis.

Next, we tried to find out whether IDO1 is essential for the protective effects of the primed MSCs. We induced experimental colitis and injected MSCs, as described in Fig. 1A. In addition, a competitive IDO inhibitor, L-1-MT (200 mg/kg), was administered by oral gavage from day 1 to day 5. Figure 6 suggests clearly that the pharmacologic IDO1 inhibition abolished the beneficial effects of the primed-MSC treatment, such as less weight loss, reduced DAI scores, and preserved colon length (Fig. 5B-D). Importantly, the mRNA expressions of Foxp3 and IDO1, which were increased by the primed-MSC treatment, were down-regulated by the IDO1 inhibition. In contrast, the expression of IL-10 was not affected by the IDO1 inhibition, implying that IL-10 was possibly secreted from monocytes rather than from Treg cells in the colon tissue of the experimental IBD, or that other mediators besides IDO1 might be involved in the IL-10 production (Fig. 5E). These results altogether suggest that IDO1 is an important mediator of the poly(I:C)-primed MSCs in promoting intestinal epithelial recovery and suppressing inflammatory responses.

**Discussion**

Several clinical trials using MSCs have reported that they have been safe and effective treatments for IBD until now. However, each trial used different procedures for tissue harvest, MSC isolation, and cell culture. The routes and timing of MSC administration were also diverse. This aspect made the obtained results frequently inconsistent and unimpressive (25). The cell priming (also referred to as pre-conditioning) is one of the most studied approaches to overcome this problem and has been known to improve the immune-modulatory attributes of MSCs. Previous studies demonstrated that MSC priming with pro-inflammatory cytokines, such as IFN-γ and TNF-α, increased the secretion of anti-inflammatory cytokines (IDO1, PGE2, TGF-β, and hepatocyte growth factor), the expression of chemokine ligands (CXCL9, CXCL10, and CXCL11), adhesion proteins (VCAM-1 and ICAM-1), and immune checkpoint molecules, such as PD-L1 (26, 27). In experimental colitis, human IFN-γ-primed MSCs showed higher migration rates to inflammatory sites and a significant reduction of mucosal damage and inflammatory responses than did non-primed MSC (28). There is still some room for improvement in the IFN-γ priming of MSCs, because its effects can be inconsistent (29) and transient (30). A few studies have been published regarding the priming of MSCs with TLR ligands, since TLRs are involved with the immune-modulating functions of MSCs (31). Waterman and colleagues reported that TLR4-primed MSCs mostly elaborated pro-inflammatory cytokines, whereas TLR3-primed MSCs expressed mostly immunosuppressive mediators (32). We previously showed that the priming of MSCs with a TLR3 ligand, poly(I:C), in the presence of IFN-γ increased IDO1 production and increased the immunomodulatory effects of MSCs. Other TLR ligands, Pam3CSK4 (TLR1/2), peptidoglycan (TLR2), LPS (TLR4), flagellin (TLR5), FSL-1 (TLR2/6), R848 (TLR7/8), and CpG (TLR9) did not increase IDO1 expression compared to IFN-γ alone.
Therefore, we hypothesized that the poly(I:C) priming of MSCs could increase the therapeutic efficacy in an IDO1-dependent manner.

The up-regulation of IDO1 is observed not only in the experimental murine colitis but also from the clinical samples of human IBD. CD103+ gut dendritic cells can express IDO1 and support Treg conversion while suppressing Th1/Th17 differentiation to limit gut inflammation (33). In the homeostatic state, gut expression of IDO1 is low and limited to the cells of the lamina propria. However, the inflammatory cytokines, including IFN-γ, TNF-α, and IL-1β, stimulate the IDO1 expression in epithelial cells, which become a significant source of IDO1 activity in IBD. The IDO1 expression is more apparent in epithelial cells near the sites of ulceration (34). In the experimental colitis model, the administration of the TLR-9 agonist improved clinical and histological parameters via the induction of IDO1, and the inhibition of IDO1 activity abrogated the protective effects (35). Gurtner et al. also demonstrated that the administration of IDO1 inhibitor 1-DL-MT worsened the IBD activity in mice, suggesting that IDO1 down-regulated Th1 responses within the intestinal tract (36). The protective effects of IDO1 were confirmed in a mouse model of graft versus host disease. Jasperson et al. showed that IDO1−/− mice exhibited greater colitis severity, T-cell infiltration, and mortality (37). They also demonstrated that induction of IDO1 primarily in professional APCs by a TLR-7/TLR-8 agonist reduced colon injury and decreased lethality (38). IDO1 secreted from MSCs also polarized the differentiation of monocytes into IL-10-producing CD206+ M2 macrophages, which in turn promote T-cell suppression (39). In addition, IDO1 blocked the intestinal bacterial growth and mediated epithelial barrier protection induced by IL-27 (40). It is worthy of note that IDO1 expression supported epithelial proliferation independently of the effects on adaptive immunity through the activation of the Wnt/β-catenin signaling pathway in the colitis-associated cancer model (azoxymethane/DSS) (41). Taken together, IDO1 expression by APCs may be critical to suppressing inflammatory T-cell responses, whereas epithelial IDO1 activity functions to limit microbial invasion and promote epithelial repair. Further studies are required to find out which one predominantly contributes to ameliorating IBD.

**Conclusion**

In this study, we demonstrated that the poly(I:C) priming had increased the therapeutic efficacy of MSCs on DSS-induced colitis. The primed MSCs alleviated the DSS-induced pathologic changes in the colon, decreased inflammatory cytokines, and stimulated the proliferation of ISC more effectively than the unstimulated MSCs did. Additionally, the primed MSCs stimulated the differentiation of intestinal epithelial cells and the restoration of the mucosal barrier to a greater extent. Last but not least, these improvements were mediated through the IDO1 and increased Treg proportion in the inflamed site. These results suggest that the priming of MSCs through stimulating TLR3 is a promising new strategy to increase the therapeutic efficacy of MSC on IBD and might be able to solve the problems that the current MSC therapies are facing. IDO1 is a critical mediator of the poly(I:C)-primed MSC in suppressing IBD, and further studies are required to unfold its functions in gut epithelium and the surrounding microenvironment.
Abbreviations

BM, Bone marrow; COX2, Cyclooxygenase 2; DAI, Disease activity index; DSS, Dextran sulfate sodium; H&E, Hematoxylin-eosin; IBD, Inflammatory bowel disease; IDO, Indoleamine 2,3-dioxygenase; IFN, Interferon; IHC, Immunohistochemistry; IL, Interleukin; i.p., Intraperitoneally; L-1MT, L-1-methyl tryptophan; mLN, Mesenteric lymph node; MSC, Mesenchymal stromal cells; NO, nitric oxide; PD-L1, Programmed cell death ligand 1; PGE2, Prostaglandin E2; PTGES3, Prostaglandin E synthase 3; TGF, Transforming growth factor; TLR, Toll-like receptor; TNF, Tumor necrosis factor.

Declarations

Acknowledgements

Not applicable

Authors’ contributions

JYL, BSK, and CKM contributed to the research conception, experimental design, and data analysis; JYL, DBR, TWK, and GSP were responsible for the collection and assembly of data; JYL and BSK wrote the manuscript; CKM provided financial support and study materials. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

All animal experiments have been approved by the Institutional Animal Care and Use Committees of the Catholic University of Korea (Seoul, Korea), and are in compliance with the Laboratory Animal Act from the Korean Ministry of Food and Drug Safety (No.15278).

Consent for publication

Not applicable

Competing interest

The authors declare they have no competing financial and non-financial interests.
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Figures
Pharmacologic IDO1 inhibition decreased the immune-modulatory effects of the poly(I:C)-primed MSC. (A) Experimental colitis was induced, and the unstimulated or primed MSCs were administered as described in Figure 1. The competitive IDO inhibitor, L-1-MT (200mg/kg), was administered by oral gavage from day 1 to day 5. All mice were followed up daily and sacrificed on day 9 to harvest colon tissues. Representative data of two independent experiments are presented (n = 5 for each group) (B) Daily body weight. (C) Daily DAI score. (D) Colon lengths were measured on day 9. (E) Results of real-time RT-PCR from colon tissue demonstrated that the oral L-1-MT administration reversed the expression of Foxp3 and IDO1, which was increased by the primed MSC treatment. Representative data of two independent experiments are presented (n = 5 for each group). (* p < 0.05, ** p< 0.01, *** p< 0.001).
Figure 2

The poly(I:C)-primed MSCs increased the expression of colonic IDO1 and COX2. Mice with DSS-induced colitis were sacrificed on day 9 to harvest colon tissues. Representative data of two independent experiments are presented (n = 4 for each group). (A) IHC images of colon sections for IDO1 are shown. Quantification of IDO1-positive cells in control, the unstimulated MSC-treated, and the primed MSC-treated mice. (B) The colonic mRNA expression of IDO1, COX2, and PTGES3 increased more in the primed-MSC group than in other groups. (* p < 0.05, ** p < 0.01, *** p < 0.001).
Figure 3

The poly(I:C)-primed MSC expanded Treg cells in an IDO1-dependent manner. Mice with the DSS-induced colitis were sacrificed on day 9 to harvest the spleen, mLN, and colon tissue. Representative data of two independent experiments are presented (n = 4 for each group). (A) Treg (CD25+Foxp3+CD4+) proportions to CD4+ T cells in spleen and mLN significantly increased more in the primed MSC group than in the DSS control group. Difference of the Treg proportion between the primed MSC and the unstimulated MSC group was evident only in the spleen. (B) The colonic mRNA expression of Treg (Foxp3) markedly increased only in the primed MSC group. (C) T cells were negatively selected from splenocytes of B6 mice and co-cultured with the unstimulated or primed MSC in the presence of anti-CD3/CD28 antibodies. The primed MSCs increased the proportion of Treg (CD25+Foxp3+CD4+) to CD4+ T cells more effectively than the unstimulated MSC did. (D) A competitive IDO1 inhibitor, L-1-MT, reversed the Treg expansion which was induced by the primed MSC. (* p < 0.05, ** p< 0.01, *** p< 0.001).
The poly(I:C)-primed MSCs decreased inflammatory response in the spleen, mLN, and colon tissue. Mice with the DSS-induced colitis were sacrificed on day 9 to harvest the spleen, mLN, and colon tissues. Representative data of two independent experiments are presented (n = 4 for each group). (A-C) Recovered cells from spleen and mLN were subjected to the flow cytometric analysis. The infiltration of monocytes (CD11+), dendritic cells (CD11c+), and CD4+ T cells significantly decreased in the primed MSC group. (D) Results of real-time RT-PCR from colon tissue demonstrated that mRNA expression of inflammatory cytokines (IL-1β, TNF-α, and IL-6) and a chemotactic factor (MCP-1) decreased significantly in the primed MSC group. The expression of immune-modulating cytokine (IL-10) increased in the same group. (* p < 0.05, ** p < 0.01, *** p < 0.001).

Figure 4
Figure 5

The poly(I:C)-primed MSCs significantly stimulated the ISC proliferation, enterocyte differentiation, and epithelial regeneration. Mice with the DSS-induced colitis were sacrificed on day 9 to harvest colon tissues. Representative data of two independent experiments are shown (n = 4 for each group). (A) IHC images of colon sections for Ki-67 are shown. Quantification of Ki-67-positive cells in control, the unstimulated MSC-treated, and the primed MSC-treated mice. (B) Results of real-time RT-PCR showed that mRNA levels of ISC markers (Lgr5, Olfm4, and Bmi1) and the Wnt/β-catenin pathway (Axin2) were significantly increased in the primed-MSC group. (C) The mRNA expressions of Muc2 (goblet cell), Alpi (enterocyte), Chga (enteroendocrine cell), and Occludin (epithelial tight junction) significantly increased in the primed-MSC group. (D) IHC images of colon sections for lysozyme are shown. Quantification of lysozyme-expressing Paneth cells in control, the unstimulated MSC-treated, and the primed MSC-treated mice. (* p < 0.05, ** p< 0.01, *** p< 0.001).
The poly(I:C)-primed MSCs were more effective than were unstimulated MSCs in ameliorating the DSS-induced colitis in mice. (A) Experimental outline. 2.5% DSS was given to B6 mice via drinking water for six days to induce colitis. The primed or unstimulated MSCs were injected i.p. to mice on days 1 and 3. All mice were sacrificed on day 9 to harvest the spleen, mLN, and colon tissues. Results are combined from two independent experiments (normal, n = 4; DSS, n = 9; DSS + unstimulated MSC, n = 9; DSS + primed MSC, n = 9). (B) Daily body weight. (C) Daily DAI score. (D) Colon lengths. (E) H&E-stained images of colon sections, representative data of two independent experiments, are shown (n = 4 for each group). The primed MSCs significantly reduced the pathologic scores. (* p < 0.05, ** p< 0.01, *** p< 0.001).