Topical Implantation of Mesenchymal Stem Cells Has Beneficial Effects on Healing of Experimental Colitis in Rats

Yujiro Hayashi, Shingo Tsuji, Masahiko Tsujii, Tsutomu Nishida, Shuji Ishii, Hideki Iijima, Toru Nakamura, Hiroshi Eguchi, Eiji Miyoshi, Norio Hayashi, and Sunao Kawano

Departments of Clinical Laboratory Science (Y.H., T.Na., H.E., E.M., S.K.) and Gastroenterology and Hepatology (S.T., M.T., T.Ni., S.I., H.I., N.H.), Osaka University Graduate School of Medicine, Osaka, Japan; and Rinku General Medical Center, Izumisano Municipal Hospital, Osaka, Japan (S.K.)

Received January 23, 2008; accepted April 29, 2008

ABSTRACT

Mesenchymal stem cells (MSCs) are attractive cell sources in regenerative medicine. We examined the effects of topical MSCs implantation on an experimental model of inflammatory bowel disease. Putative MSCs, isolated from bone marrow aspirates of male rats by dish adherence and expanded in vitro, were characterized by flow cytometry, reverse transcription-polymerase chain reaction, enzyme-linked immunosorbent assay, and differentiation assays. Experimental colitis was induced by intraluminal instillation of 2,4,6-trinitrobenzene sulfonic acid (TNBS) in the colons of male rats. The putative MSCs and unselected fresh bone marrow cells were injected into the colonic submucosa surrounding the area exposed to TNBS. The healing process of the injury was examined macroscopically and immunohistologically. Multipotent MSCs positive for CD29 and CD90, and negative for CD31 and CD34, were implanted into colon tissue surrounding the lesion; a majority of the engrafted cells were positive for vimentin. The implantation significantly accelerated healing of the damaged mucosa compared with vehicle-injected controls. The MSCs expressed vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β1 in vitro and after the implantation. In conclusion, we found that MSCs were successfully topically implanted in the colon and that they were associated with accelerated healing of TNBS-induced colitis. The beneficial effects of the MSCs might be mediated, at least in part, by their ability to differentiate into colonic interstitial cells and by their ability to provide VEGF and TGF-β1 to the injured area.

Despite many advances in basic (Baumgart and Carding, 2007) and clinical science (Baumgart and Sandborn, 2007), treatment of inflammatory bowel diseases (IBD) is far from satisfactory. Several studies suggest that immunological changes in lymphocytes are responsible for both the development and healing of IBD (Bouma and Strober, 2003). Myeloid cell function was also found to be important in a cohort of patients with Crohn’s disease (Dieckgraefe and Korzenik, 2002). In contrast, emerging data suggest nonhematopoietic cells play a critical role in the development and healing of IBD (Olson et al., 2006). For example, the expression of Toll-like receptors in mucosal epithelia is altered in IBD (Cario and Podolsky, 2000). In addition, mucosal mesenchymal and endothelial cells play decisive roles through their interactions with immune cells (Fiocchi et al., 2006).

Other studies have shown that bone marrow-derived cells contribute to the healing of gastrointestinal wounds in humans (Okamoto et al., 2002; Matsumoto et al., 2005) and animals (Brittan et al., 2002, 2005; Komori et al., 2005a,b; Bamba et al., 2006; Hayashi et al., 2007; Khalil et al., 2007). Moreover, bone marrow-derived cells are recruited more efficiently to the injured gut than to a healthy gut, suggesting a rescue response for assisting with the recovery from damage (Komori et al., 2005a,b). Previous studies showed the transplantation of wild-type bone marrow in mutant mice lacking interleukin (IL)-10 apparently ameliorated an inflamed bowel (Bamba et al., 2006). Furthermore, the nonmyeloablative transplantation of immortalized cells in an experimental model of IBD promoted tissue regeneration (Khalil et al., 2007). However, it is still unclear what types of cells are able to exert these beneficial effects and thus which types would be most appropriate for IBD treatment.
In contrast to hematopoietic stem cells, bone marrow-derived mesenchymal stem cells (MSCs) are easily isolated from the bone marrow and have been extensively studied for their ability to differentiate into various cell types in vitro (Peister et al., 2004). Due to their multipotency, MSCs are an attractive cell source for regenerative medicine. The aims of the present study were to examine the effects of topical implantation of bone marrow-derived MSCs on experimental colitis and to characterize phenotypes of the implanted MSCs. We used rats bearing colitis as an experimental model for investigating the beneficial effects of MSC implantation.

### Materials and Methods

**Experimental Animals.** Male Sprague-Dawley rats were purchased from Japan SLC, Inc. (Shizuoka, Japan). All animal studies were reviewed and approved by the institutional committee on use and care of animals at Osaka University Graduate School of Medicine.

**Preparation of Bone Marrow-Derived Dish-Adherent Cells (Putative MSCs).** Bone marrow cells were collected by flushing the bone shafts of the femurs and tibias of male rats with Medium 199 (Invitrogen, Carlsbad, CA) supplemented with 5% fetal calf serum (JRH Biosciences, Lenexa, KS) and 1% antibiotics and antymycotics (Invitrogen) using a 21-gauge needle. After filtering the cells through a 50-μm nylon mesh and washing twice with Medium 199, the bone marrow cells were cultured in α-minimal essential medium (Invitrogen) supplemented with 10% heat-inactivated fetal calf serum, 200 U/ml penicillin G, and 200 μg/ml streptomycin sulfate at a density of 1 × 10⁶ cells per plastic dish (10 cm in diameter; Corning International, Tokyo, Japan). These cells made up the unselected bone marrow cells (BMCs). To isolate putative MSCs, after 24 h of culture, nonadherent cells were removed by washing the cells with phosphate-buffered saline (PBS), and adherent cells were maintained; the culture medium was replaced twice a week. The dish-adherent MSC population was expanded in 3 to 5 passages after the initial plating.

**Characterization of the Putative MSCs.** Expression of surface markers was examined by flow cytometry using FACScan (Immunocytochemistry Systems, San Jose, CA). Cells were incubated with fluorescein isothiocyanate-conjugated mouse monoclonal antibodies against CD31 (clone MCA1334FA; Serotec, Oxford, UK), rat CD34 (clone IC-105; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), CD45 (clone OX-1; BD Biosciences, San Jose, CA), CD29 (clone MCA1334FA; Serotec, Oxford, UK), rat CD34 fluorescein isothiocyanate-conjugated mouse monoclonal antibodies (BioLegend, San Diego, CA), CD90 (clone OX-7; BD Biosciences, San Jose, CA), and CD105 (clone MCA105; Serotec, Oxford, UK). The cells were labeled with a fluorescent cell linker, PKH67 (Sigma-Aldrich, St. Louis, MO), and vimentin (clone V9; Santa Cruz Biotechnology, Inc.). Isotype-identical antibodies (clone 2E1; Medical and Biological Laboratories Co., Ltd., Nagoya, Japan) served as controls.

The expression of IL-10 and IL-12 growth factors typically found in MSCs was explored by reverse transcription-polymerase chain reaction (RT-PCR) using the GeneAmp PCR System 9600 (PerkinElmer Applied Biosystems, Roissy, France) and Ready-To-Go PCR Beads (GE Healthcare, Little Chalfont, Buckinghamshire, UK). To monitor cDNA synthesis efficiency, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The PCR primers were designed using Primer premier 5.0. The PCR products were electrophoresed in 2% agarose gels containing ethidium bromide in 1× Tris acetate-EDTA buffer.

To investigate whether MSCs produced VEGF, we measured VEGF levels in the MSC-conditioned medium (1 × 10⁶ cells in 10-cm dish cultured for 48 h). VEGF and TGF-β1 were measured using an enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer’s protocol (VEGF immunoassay and TGF-β1 immunoassay; R&D Systems, Minneapolis, MN).

### Table 1

<table>
<thead>
<tr>
<th>mRNA</th>
<th>PCR Primers (Forward and Reverse)</th>
<th>PCR Cycles</th>
<th>PCR Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>5’-AGT CTT GCC AAT GTG GAC TC-3’</td>
<td>34</td>
<td>515</td>
</tr>
<tr>
<td></td>
<td>5’-GCC AGT GGA TTC TCA TCT TG-3’</td>
<td>25</td>
<td>396</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>5’-GGCTACTGCCATCCACCTTGGC-3’</td>
<td>29</td>
<td>392</td>
</tr>
<tr>
<td></td>
<td>5’-GGGTTGACCTTCTGTTGACCCTG-3’</td>
<td>30</td>
<td>389</td>
</tr>
<tr>
<td>IL-10</td>
<td>5’-TGG TAT GCC GTC GTG TTT G-3’</td>
<td>35</td>
<td>542</td>
</tr>
<tr>
<td></td>
<td>5’-AGG TAG TCC AGG ATG ACG TGG TT-3’</td>
<td>28</td>
<td>308</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5’-TCC CTC TCT AAC ATT GTG AGC AA-3’</td>
<td>29</td>
<td>392</td>
</tr>
<tr>
<td></td>
<td>5’-AGA TCC ACA ACG GAT ACA TT-3’</td>
<td>28</td>
<td>308</td>
</tr>
</tbody>
</table>

bp, base pairs.

**Induction of TNBS Colitis.** Colitis was produced in 7-week-old Sprague-Dawley rats using the method of Uchida and Mogami (2005), with minor modifications. In brief, the rats were laparotomized under sevoflurane anesthesia (Maruishi Pharmaceutical Co., Ltd., Osaka, Japan). The proximal colon was clamped with ringed forceps that had a 10-mm inner diameter. An ethanol solution (35%; 0.2 ml) containing 0.15 M TNBS (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was injected into the proximal colon lumen via a 29-gauge needle. The clamp was maintained for 2 min to allow colon injury to develop (Fig. 1).

**Cell Implantation.** As a preliminary experiment, we examined the influences of transplanted cell number on ulcer healing. Because the effect of 1 × 10⁶ cells was modest for the primary implantation we used 1 × 10⁷ bone marrow-derived cells. Before implantation, the cells were labeled with a fluorescent cell linker, PKH67 (Sigma-Aldrich) (Melincoff et al., 1988), to enable tracing the cells in vivo. The PKH67-labeling procedure for adult somatic stem cells has been described previously in detail (Askenasy and Farkas, 2002; Gregaard et al., 2007). In brief, 1 × 10⁷ cells were incubated in 1 ml of diluent with PKH67 for 5 min as suggested by the manufacturer. The cells were collected by centrifugation (400g for 10 min), resuspended, and washed twice with PBS. The recovery rate of this procedure was 80 to 90% viable cells. Immediately after the TNBS-induced colon injury, the rats were randomized, and they received injections with 1 × 10⁷ putative MSCs in 100 μl of PBS per animal or with 100 μl of PBS alone. Submucosal injections were given into the colonic wall at three points surrounding the damaged area (Fig. 1). In addition, another group of rats was randomized into two groups, and they received...
injections with $1 \times 10^7$ unselected BMCs in PBS (100 µl) or with 100 µl of PBS alone. The abdomen was closed and the animal was allowed to recover from the procedure with free access to tap water and standard pellet chow.

Assessment of TNBS-Induced Colonic Injury. The systemic influence of colitis was first evaluated by weighing each animal daily. On day 6, the rats were anesthetized and sacrificed for macroscopic and microscopic analyses of the induced colonic injury. The chest and abdominal wall were opened with a midline incision, and the inferior vena cava was cut for exsanguination. The chest and abdominal wall was examined except for the granulation tissue, which was too fragile for processing. All images were captured by a digital imaging system.

Fig. 1. Schematic drawing showing the induction of colitis with a TNBS injection and the topical implantation of MSCs.

For immunohistochemical analysis, cryostat sections (5 µm in thickness) were incubated at 37°C for 1 h in 3% normal goat serum to prevent putative non-specific binding to mouse immunoglobulins. Then, the sections were incubated at 4°C for 24 h with primary antibodies against vimentin [clone V9; YLEM, Avezzano (AQ), Italy], α-SMA (clone 1A4; Dako UK Ltd., Ely, Cambridgeshire, UK), desmin (clone RD-301; Santa Cruz Biotechnology, Inc.), von Willebrand factor (A0082; Dako UK Ltd.), Ki-67 (YLEM), VEGF (clone A-20; Santa Cruz Biotechnology, Inc.), and TGF-β1 (clone TB21; Oxford Biotechnology, Co., Ltd., Oxford, UK). After three washes for 5 min each in PBS, the sections were incubated with the appropriate secondary antibodies labeled with rhodamine red. To visualize cell nuclei, the sections were stained with 4',6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA), and then they were observed under a fluorescence microscope (Nikon Eclipse TE2000-U; Nikon, Tokyo, Japan) or a confocal laser-scanning microscope with appropriate filters (LSM510; Carl Zeiss, Oberkochen, Germany). The colon wall was examined except for the granulation tissue, which was too fragile for processing. All images were captured by a digital imaging system.

Statistical Analysis. Flow cytometry, RT-PCR, and differentiation assays were performed at least in triplicate, and typical results were presented. Parametric data are expressed as mean ± S.D., and they were analyzed by a two-tailed Student’s t test or analysis of variance followed by multiple comparisons of the means. p < 0.05 was considered statistically significant.

Results

Characterization of the Dish-Adherent Putative MSCs. The bone marrow-derived putative MSCs isolated by dish adherence and expanded as described above were characterized by flow cytometry, RT-PCR, ELISA, and differentiation assay. More than 97% of the cells expressed CD29 (99.1 ± 1.3%) and CD90 (97.6 ± 4.1%). In contrast, there were virtually no CD31-positive endothelial cells (0.2 ± 0.1%) or CD34-positive immature hematopoietic cells (0.1 ± 0.1%). In addition, less than 4% of the cells expressed CD45 (3.4 ± 2.8%). At passage 5, the cells readily differentiated into Alizarin red-positive mineralizing cells (osteocytes) or oil red O-positive adipocytes when incubated in the respective differentiation media for 21 days. Therefore, the adherent cells maintained the undifferentiated MSCs phenotypes of multipotent, nonhematopoietic cells.

MSCs secrete a variety of growth factors and cytokines (Liu et al., 2006). The RT-PCR study demonstrated that the cells at passage 5 expressed VEGF and TGF-β1 mRNAs (Fig. 2). However, the cells did not express IL-10 mRNA (Fig. 3). After 48 h in culture, MSCs secreted more VEGF and TGF-β1 than unselected BMCs. The phenotypes of the bone marrow-derived dish-adherent MSCs are summarized in Table 2.

Effects of MSC Implantation on Development and Healing of TNBS-Induced Colitis. As a preliminary experiment, we examined the effects of i.v. administration of PKH67-labeled MSCs ($1 \times 10^7$ cells) on TNBS-induced colitis in rats. However, i.v. administration of MSCs did not mitigate the colonic damage and body weight loss induced by TNBS. Furthermore, PKH67-labeled MSCs accumulated in lung rather than in colonic tissues, including those surrounding the TNBS injury (data not shown).

As expected, body weight increased gradually in untreated rats. In contrast, rats treated with luminal TNBS and submucosal PBS injections (TNBS + PBS) were exhausted, and they exhibited a significant loss of body weight. Significant and continuous weight loss was also observed in rats that received luminal TNBS and implantation of unselected BMCs (TNBS + BMCs). However, the rats that received luminal TNBS and MSCs implantation (TNBS + MSCs) exhibited a transient loss of body weight followed by a recovery. The body weight was significantly higher in the TNBS + MSCs group than in the TNBS + PBS group on days 3 to 6 (Fig. 4A). It is interesting to note that transplantation of freshly isolated, unselected BMCs ($1 \times 10^7$ cells) did not mitigate the body weight loss induced by TNBS injections (Fig. 4B).

On day 3, the areas of damaged colon tissue were comparable between the TNBS + PBS and the TNBS + MSCs groups ($104 ± 11.31$ and $88.75 ± 9.8$ mm$^2$, respectively; $p = 0.062$). On day 6, the lesion areas were $70.36 ± 9.48$ and
TABLE 2
Phenotypes of the putative MSC isolated by dish adherence in vitro

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Physiologic Meaning</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD29</td>
<td>β1-Integrin</td>
<td>+,p&lt;0.05</td>
</tr>
<tr>
<td>CD31 (PECAM-1)</td>
<td>Endothelial cell marker</td>
<td>-a</td>
</tr>
<tr>
<td>CD44</td>
<td>L-Selectin ligand</td>
<td>-a</td>
</tr>
<tr>
<td>CD45 (LCA)</td>
<td>Leukocyte common antigen</td>
<td>-a</td>
</tr>
<tr>
<td>CD90</td>
<td>Thy-1.1 antigen</td>
<td>+,p&lt;0.05</td>
</tr>
<tr>
<td>IL-10</td>
<td>Th2 cytokine</td>
<td>-b</td>
</tr>
<tr>
<td>VEGF</td>
<td>Angiogenic factor</td>
<td>+,p&lt;0.05</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>Transforming growth factor-β1</td>
<td>+,p&lt;0.05</td>
</tr>
</tbody>
</table>

LCA, leukocyte common antigen; PECAM-1, platelet/endothelial cell adhesion molecule-1; Th2, T-helper 2.

*p<0.05 versus control group (TNBS + PBS).

Material and Methods. Table 2 shows the results of cell adhesion molecule expression by RT-PCR analysis of IL-10 mRNA in bone marrow-derived MSCs after 5 passages in vitro. Pure water (H₂O) and splenocytes serve as negative and positive controls, respectively. M, molecular marker. GAPDH was the control for assay efficiency.

Phenotypes of MSCs after Implantation. Fluorescence microscopy showed a heterogeneous distribution of green fluorescent (PKH67)-labeled MSCs within the submucosa surrounding the colon injury. Three and 6 days after implantation, MSCs were successfully engrafted into the appropriate submucosal layers (Fig. 6, b–e). Immunofluorescence microscopy demonstrated that approximately 50% of transplanted MSCs were positive for the interstitial lineage marker vimentin, and only a small proportion were positive for the smooth muscle cell/myofibroblast marker α-SMA and the smooth muscle marker desmin (Fig. 7A). Furthermore, PKH67-positive, α-SMA-positive MSCs were spindle-shaped, indicating that a minor population of MSCs had differentiated into myofibroblasts. The percentages of transplanted MSCs that expressed vimentin, α-SMA, and desmin were 47.2 ± 14.6, 6.1 ± 3.2, and 7.3 ± 4.9, respectively (Fig. 7B).

43.7 ± 10.15 mm² in the TNBS + PBS and TNBS + MSCs groups, respectively (p < 0.05). MSC implantation significantly accelerated healing of the TNBS-induced mucosal injury compared with vehicle-treatment (Fig. 5, A and B). In contrast, BMC implantation did not significantly change the healing rate of the TNBS-induced mucosal injury compared with vehicle treatment (Fig. 5C). On day 6, the lesion areas were 69.6 ± 22.92 and 56.8 ± 21.48 mm² in the TNBS + PBS and TNBS + BMCs groups, respectively (p = 0.38).

Histological analysis showed that, as reported originally (Uchida and Mogami, 2005), TNBS caused ulcerative colon injury penetrating toward the proper muscle layer. On day 3, the lesions were characterized by edema, epithelial exfoliation, and infiltration of leukocytes. The ulcer margins were clear, and they did not yet develop immature epithelium in any of the groups. On day 6, the ulcer margins had an extension of regenerated epithelia in all the groups. The proper muscle layer was disrupted at the site of ulceration throughout the study period. However, on day 6, the depth of the tissue defect was shallower in rats with MSC implantation than in controls (Fig. 6, a and b).

Phenotypes of MSCs after Implantation. Transformed MSCs that expressed vimentin, α-SMA, and desmin were differentiated into myofibroblasts. The percentages of transplanted MSCs that expressed vimentin, α-SMA, and desmin were 47.2 ± 14.6, 6.1 ± 3.2, and 7.3 ± 4.9, respectively (Fig. 7B). These results suggest that MSCs were able to differentiate into interstitial lineage cells, fibroblasts, and myofibroblasts. It should be noted that the colon also contained PKH67-negative host cells expressing vimentin, α-SMA, or desmin. Moreover, there was neither endothelial cell positive for PKH67 and von Willebrand factor nor proliferating cell positive for PKH67 and Ki-67 in the colon (data not shown). Furthermore, when they were implanted into the normal colon (non-TNBS), MSCs partly differentiated into colonic interstitial lineage cells 6 days after the implantation (Fig. 8). These results suggested that the differentiation of engrafted MSCs in the colon did not depend on the colonic inflammation. We also found expression of VEGF and TGF-β1 in colon mucosa after MSC implantation. The RT-PCR study showed that the expression of VEGF and TGF-β1 mRNAs in the colon tissues was not apparently different between the TNBS + PBS group and TNBS + MSCs groups 6 days after the implantation (data not shown). However, VEGF and TGF-β1 expression in the colon tissues was significantly greater in the MSC group than in the control group (Fig. 9).
TGF-β1 immunostaining was observed in PKH67-positive MSCs surrounding the lesion area on day 3 (Fig. 9) and day 6 (Fig. 10A), suggesting the implanted MSCs secreted VEGF and TGF-β1 locally. The percentages of MSCs that expressed VEGF and TGF-β1 were 27.6 ± 16.6 and 26.5 ± 15.5, respectively, 6 days after the implantation (Fig. 10B). It is noteworthy that some MSCs expressing VEGF or TGF-β1 were not necessarily thin-spindle-shaped. Rather, the majority of MSCs expressing VEGF or TGF-β1 were oval- or thick-spindle-shaped. VEGF and TGF-β1 were also expressed in PKH67-negative, spindle-shaped cells; these results indicated that host-derived interstitial lineage cells might also be sources of TGF-β1 and VEGF in the colon.

**Discussion**

In this study, CD29-positive, CD90-positive, CD31-negative, and CD34-negative multipotent MSCs were isolated...
from bone marrow cells by dish adherence. The bone marrow-derived MSCs were implanted and survived within the colonic wall. Histological analyses showed that a majority of the engrafted MSCs were positive for vimentin (the major subunit protein of the intermediate filaments of mesenchymal cells: an interstitial lineage marker). However, only small amounts of the engrafted MSCs were positive for either α-SMA (a marker for myofibroblast/smooth muscle) or desmin (type III intermediate filaments: a marker for myocytes, smooth muscles, and other muscle cells). These results suggest that MSCs had a tendency to differentiate into interstitial or stromal lineage cells. The labeled cells were well distributed within the site of injection and did not form tumors or capsules. Therefore, the implantation of MSCs seemed to be safe; however, the long-term fate of the engrafted MSCs remains to be investigated.

The size of the TNBS-induced colon injury by day 3 was comparable between the rats that received MSC implantation and those given PBS. However, by day 6, the injury was significantly smaller in the TNBS + MSCs group than the TNBS + PBS group. Histological analyses also showed that by day 6, the depth of the injury was significantly shallower in the TNBS + MSCs group than in the TNBS + PBS group. These results show that topical implantation of MSCs accelerated the healing of a colon injury. In an earlier period, (i.e., on day 3 after the colitis induction) engrafted MSCs also differentiated into colonic interstitial lineage cells and produced VEGF and TGF-β1. These results suggest that the implantation at least partially ameliorated TNBS-induced colonic inflammation.

A previous study reported that transplantation of wild-type bone marrow cells ameliorated spontaneous colitis in IL-10 null mice (Bamba et al., 2006), suggesting an important role of this cytokine in the development and healing of colon inflammation. However, in the present study, MSCs cultured in vitro did not express IL-10 mRNA. Therefore, it is unlikely that the implantation accelerated healing of the TNBS-induced colon injury by providing IL-10. In contrast, our RT-PCR and ELISA results clearly demonstrated that MSCs cultured in vitro expressed and secreted both VEGF
Furthermore, fluorescence immunohistochemistry showed that the engrafted MSCs also expressed these growth factors in the injured colon in vivo. TGF-β1 and VEGF are known to be produced in various types of cells, and to play important roles in gastrointestinal wound healing. TGF-β1 has potent effects on gastrointestinal mucosal integrity, wound repair (Beck et al., 2003), angiogenesis (Daniel and Abrahamson, 2000), and the regulation of acquired (Letterio and Roberts, 1998) and innate immunity (Meneghin and Hogaboam, 2007). In human IBD, TGF-β1 expression was reported to be enhanced in gut mucosa (Babyatsky et al., 1996). In a mouse model of IBD, the loss of TGF-β1 signaling contributed to intestinal injury (Hahm et al., 2001). In human IBD, TGF-β1 expression was reported to be enhanced in gut mucosa (Babyatsky et al., 1996). In a mouse model of IBD, the loss of TGF-β1 signaling contributed to intestinal injury (Hahm et al., 2001).

TGF-β1 has potent effects on gastrointestinal mucosal integrity, wound repair (Beck et al., 2003), angiogenesis (Daniel and Abrahamson, 2000), and the regulation of acquired (Letterio and Roberts, 1998) and innate immunity (Meneghin and Hogaboam, 2007). In human IBD, TGF-β1 expression was reported to be enhanced in gut mucosa (Babyatsky et al., 1996). In a mouse model of IBD, the loss of TGF-β1 signaling contributed to intestinal injury (Hahm et al., 2001). In human IBD, TGF-β1 expression was reported to be enhanced in gut mucosa (Babyatsky et al., 1996). In a mouse model of IBD, the loss of TGF-β1 signaling contributed to intestinal injury (Hahm et al., 2001).

VEGF is a growth factor that stimulates recruitment of both leukocytes and vascular endothelial cells. The importance of VEGF in wound healing in the gut has been described in detail (Tarnawski, 2005). Therefore, our results suggest that both MSCs may not differentiate to myofibroblasts or to smooth muscle cells within 6 days after implantation. However, MSC-derived TGF-β1 may play a role in wound contraction via its effects on host myofibroblasts and other cells; this remains to be investigated in a long-term study.

VEGF is a growth factor that stimulates recruitment of both leukocytes and vascular endothelial cells. The importance of VEGF in wound healing in the gut has been described in detail (Tarnawski, 2005). Therefore, our results suggest that both MSCs may not differentiate to myofibroblasts or to smooth muscle cells within 6 days after implantation. However, MSC-derived TGF-β1 may play a role in wound contraction via its effects on host myofibroblasts and other cells; this remains to be investigated in a long-term study.
ing process of gut injury. Bone marrow-derived cells are temporally and efficiently recruited into gastrointestinal tissue during the healing process of chemical injury (Komori et al., 2005a,b). The involvement of the bone marrow-derived cells into gastrointestinal tissue reduces after the damage was completely healed, suggesting that the bone marrow-derived cells participate positively in wound healing. Brittan et al. (2005) reported that bone marrow cells were involved in neovasculogenesis in the colon after TNBS-induced colitis in mice. Furthermore, Khalil et al. (2007) reported that i.v. infusion of CD34-negative cells immortalized with simian virus 40 large-T antigen promoted tissue regeneration and involved in neovasculogenesis in murine dextran sulfate sodium-induced colitis (Khalil et al., 2007). However, in the present study using nonimmortalized MSCs, we did not find any involvement of MSCs in neovasculogenesis 6 days after the induction of TNBS-induced colitis. These results in rats are consistent to the findings of Sato et al. (2005) who reported that there was no evidence of differentiation of human MSCs into CD31-positive endothelial cells in the rat liver. Although the reasons for these discrepancies remain unclear, the discrepancy between these studies might be due to differences in the methods used to transplant stem cells, differences in the experimental animals used, differences in the phenotype of stem cells used and different methods used for tissue analyses.

Finally, the present data demonstrate that MSCs can be engrafted safely and successfully by topical injection. The preliminary study showed that nonimmortalized CD29-positive, CD90-positive, CD31-negative, CD34-negative, and CD45-negative MSCs were trapped mainly in lung, and they were hardly found in the injured colon. Furthermore, the i.v. administration of $1 \times 10^7$ MSCs was insufficient to accelerate healing of TNBS-induced injury, whereas the topical implantation of the same number of MSCs was sufficient to obtain the beneficial effects. The topical injection enables to deliver larger amount of nonimmortalized MSCs than i.v. administration proposed by Khalil et al. (2007). These findings are clinically relevant, because the gastrointestinal tract is easily accessible with endoscope technology. Active IBD, i.e., Crohn's disease and ulcerative colitis, often displays deep lesions that can be identified under colonoscopy or enteroscopy. Endoscopic injection has been used in hemostatic treatments against acute gastrointestinal bleeding and in the nonsurgical removal of colon adenomas and early stage carcinomas (Sivak, 2000). We have shown that topical injection can be applied to engraftment of MSCs into a target area.

In conclusion, we have isolated and expanded MSCs in vitro and successfully implanted them into inflamed gut tissue in rats in vivo. The engrafted MSCs survived and accelerated healing of TNBS-induced colitis. After the implantation, the MSCs became potential sources of VEGF and TGF-β1, angiogenic and immunomodulating factors, in colon tissues. Minor populations of the MSCs differentiated into myofibroblasts, fibroblasts, and other interstitial lineage cells; these may also have participated in healing TNBS-induced colitis. Thus, the topical implantation of MSCs is a potentially safe and useful strategy for the treatment of IBD.

References


