Hepatocyte growth factor ameliorates dextran sodium sulfate-induced colitis in a mouse model by altering the phenotype of intestinal macrophages

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Abstract. Hepatocyte growth factor (HGF) serves key roles in cell motility, proliferation and immunoregulatory functions. However, the effect of HGF on macrophages is unclear. The present study aimed to elucidate the effect of HGF on the phenotypic alterations of intestinal lamina propria mononuclear cells (LPMCs). Colitis was induced in a mouse model using dextran sodium sulfate (DSS). Subsequently, LPMCs were isolated from the mice with chronic colitis and the expression levels of cytokine-encoding genes in the LPMCs were determined. CD11b-positive macrophages isolated from LPMCs were cultured with HGF, and alterations in the levels of M1 or M2 markers were evaluated by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and flow cytometry. In addition, the cytokine levels were assessed using RT-qPCR and ELISA. HGF shifted the phenotype of macrophages from M1 to M2-like, as determined by increased mRNA expression levels of arginase-1, CD206 and IL-10, and reduced mRNA expression levels of CD86 and IL-6 in mice with DSS-induced colitis. Moreover, HGF could ameliorate DSS-induced colitis owing to its immunosuppressive effect on immune cells. These findings indicated that HGF treatment may not only promote the regeneration of epithelial cells but also lead to tissue repair by phenotypic alteration of M1 macrophages to M2-like macrophages.

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Abbreviations: IBDs, inflammatory bowel diseases; HGF, hepatocyte growth factor; LPMCs, lamina propria mononuclear cells; IL, interleukin; iNOS, inducible nitric oxide synthase; TNF-α, tumor necrosis factor-α; Arg-1, arginase-1; TGF-β1, transforming growth factor-β1; RT-qPCR, reverse transcription-quantitative polymerase chain reaction

Key words: HGF, IBD, colitis, macrophage, phenotypic alteration

Introduction

Inflammatory bowel diseases (IBDs) such as ulcerative colitis and Crohn's disease are refractory disorders characterized by the recurrence of gastrointestinal inflammation (1). Several studies have revealed that intestinal dysbiosis leads to pro-inflammatory immunological responses and induces gastrointestinal inflammation (2-4). Intestinal macrophages influence the homeostasis of the gastrointestinal tract and help maintain a balance between the commensal intestinal microbiota and the host, but intestinal dysbiosis promotes macrophage dysfunction. Therefore, disorders of intestinal macrophages may invoke immune responses against commensal bacteria, leading to the development of chronic intestinal inflammation; thus, they play an important role in the pathogenesis of IBDs (5-7). Macrophages are innate immune cells involved in homeostasis, immune response, inflammation, and regeneration and resolution of tissues (8). Macrophage polarization states are mainly divided into the following two types: M1, pro-inflammatory or classically activated; and M2, anti-inflammatory or alternatively activated (9). M2 macrophages are capable of anti-inflammatory responses and tissue repair. Macrophages dynamically alter their phenotype, switching from the M1 to M2 phenotype during tissue repair (10). However, little is known about the factors promoting this conversion during tissue repair. Previous reports have shown that hepatocyte growth factor (HGF) signaling affects cellular responses in macrophages (11-13).

HGF was originally purified from the plasma of patients with fulminant hepatic failure as a protein that stimulates DNA synthesis in hepatocytes (14). Increased HGF expression is observed after experimental hepatic, renal, cardiac, or pulmonary injury and is associated with tissue repair (15). The ligand HGF binds to the tyrosine-protein kinase Met (c-MET), a single-pass transmembrane, disulfide-linked a/b heterodimer receptor (16). HGF-MET signaling promotes angiogenesis, cellular motility, growth, invasion, morphological

differentiation, embryological development, tissue regeneration, and wound healing in various organs (17). Previous studies have shown that HGF also ameliorates experimental colitis by accelerating intestinal epithelial regeneration, promoting cell proliferation, and preventing apoptosis. HGF aids in intestinal repair by mainly contributing to intestinal epithelial regeneration (18-20). However, a recent study has revealed that post-injury intestinal repair is regulated by the anti-inflammatory processes of macrophages (21,22); thus, further analyses are required to clarify the effects of HGF on intestinal macrophages.

In this study, we investigated the effect of HGF on intestinal immune cells, particularly intestinal macrophages using a dextran sodium sulfate (DSS)-induced colitis mouse model.

Materials and methods

Mice. Specific-pathogen-free C57BL/6J mice (6-8-week-old) were sourced from Charles River Laboratories, Japan. The mice were maintained under standard conditions (24˚C, 50-60% humidity, and a 12 h light/dark cycle) with *ad libitum* access to standard mouse chow diet (LabDiet Autoclavable Rodent Diet 5010, #0006524, PMI Nutrition International). We monitored body and stool conditions and measured the body weight of the mice each day. The mice were euthanized via cervical dislocation after being anesthetized with intraperitoneal administration of ketamine (75 mg/kg) and medetomidine (1 mg/kg), and colonic tissues were collected following euthanasia. Animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Kagoshima University (Permit no: MD19057), and the experiments were performed in accordance with the committee guidelines for animal experiments.

Induction and assessment of colitis. The DSS-induced colitis mouse model is an established experimental model that enables the investigation of various colitis symptoms, including diarrhea, weight loss, bloody stool, mucosal ulceration, and shortening of the large intestine. In this study, acute experimental colitis was induced in mice using 3% DSS (FUJIFILM Wako Pure Chemical Industries, Ltd.; average molecular weight: 5,000). Control non-colitis mice were administered distilled water. Colon tissue was collected 5 days after DSS treatment for pathological and histological analyses.

After intraperitoneal administration of 200 μg (10 mg/kg) HGF or phosphate-buffered saline (PBS), as a vehicle for HGF, to DSS-induced colitis mice or untreated control mice for 5 day, body weight, colon length, and disease activity index (DAI) based on clinical scores for weight loss, stool consistency, and bleeding (each score from 0 to 4, and the parameter values were summed, maximum score 12) were measured (23), and the colon tissues were extracted for the assessment of pathological score and gene expression assay. This colitis model is characterized by the absence of severe epithelial defects in the colon.

Histological assessment and evaluation. Colon tissue was fixed in 10% phosphate-buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The stages of colitis were evaluated via blinded histopathological analysis, according to previously described morphological criteria, namely epithelial properties, depth of inflammation, and degree of ulceration (24). Epithelial properties were scored as follows: 0, intact crypt; 1, loss of the basal one-third of the crypt; 2, loss of the basal two-thirds of the crypt; 3, loss of the entire crypt, with intact surface epithelium; and 4, loss of the entire crypt and surface epithelium (erosion). The depth of inflammation was scored as follows: 0, no infiltration; 1, crypt base; 2, mucosa; 3, submucosa; 4, submucosa (extensive). The degree of ulceration was scored as follows: 0, none; 2, positive; 4, positive (extensive). A histological score ranging from 0 to 12 was assigned to each histological specimen.

Isolation of murine colonic lamina propria mononuclear cells (LPMCs). LPMCs were isolated from the large intestine of the mice according to a previous report (23). Briefly, the colon samples were cut into small pieces in a Ca^{2+}/Mg^{2+}-free 1.5% Hank's balanced salt solution (1.5% HBSS), washed three times at 37˚C with PBS, and incubated with HBSS containing 1 mM dithiothreitol (#15-508-031, Invitrogen; Thermo Fisher Scientific, Inc.) and 5 mM EDTA (#15-575-020, Invitrogen) for 30 min at 37˚C to remove the epithelial layer. The samples were then placed in a digestion solution containing 1.5% HBSS, 1.0-3.0 mg/ml collagenase A (#11-088-793-001, Roche Diagnostics GmbH), and 0.1 mg/ml DNase I (#11-284-932-001, Roche Diagnostics GmbH) for 1-2 h at 37˚C. The samples were passed through a 100-μm strainer, transferred to a 50-ml Falcon tube, and centrifuged 200 x g, for 5 min at 4˚C. The supernatant was washed, and the pellets were resuspended in 40% Percoll and overlaid on a 75% Percoll fraction. Mononuclear cells were collected at the interphase, washed, and resuspended in RPMI-1640 medium (#11875-093, Life Science Products Inc.) containing 10% FBS and 1% penicillin/streptomycin. After isolation, the LPMCs were seeded in 12-well plates ($1x10^6$ cells per well) and treated with 10 ng/ml human HGF (E3112; EA Pharma Co., Ltd.) for 24 h in RPMI-1640 medium containing 10% FBS and 1% penicillin/streptomycin.

Cell separation of LPMCs by magnetic-activated cell sorting (MACS). Colonic LPMCs were separated using MACS. LPMCs with specific CD (cluster of differentiation) antibodies were magnetically labeled with their respective magnetic beads (CD11b; #130-049-601, Miltenyi Biotec). The cell suspension was loaded onto a MACS LS Column (#130-042-401, Miltenyi Biotec), which was placed in the magnetic field of a MACS separator (#130-042-301, Miltenyi Biotec). The MACS separation buffer contained BSA (#A9576, Miltenyi Biotec) at a final concentration of 0.5%. After the cells were separated, they were analyzed for gene expression.

Gene expression analysis. Total RNA was extracted from the colon tissues or LPMCs using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was performed using SYBR (Applied Biosystems). After detection of the threshold cycle for each mRNA in each sample, relative mRNA concentrations were calculated and normalized to that of β-actin. PCR conditions included an initial holding

Table I. Primer sequences used in this study.

Gene	Forward, 5'-3'	Reverse, 5'-3'
iNOS	CAAGCTGAACTTGAGCGAGGA	TTTACTCAGTGCCAGAAGCTGGA
CD86	ATATGACCGTTGTGTGTGTTCTGGA	AGGGCCACAGTAACTGAAGCTGTAA
Arg-1	AGCTCTGGGAATCTGCATGG	ATGTACACGATGTCTTTGGCAGATA
CD206	AGCTTCATCTTCGGGCCTTTG	GGTGACCACTCCTGCTGCTTTAG
TNF-α	TATGGCCCAGACCCTCACA	GGAGTAGACAAGGTACAACCCATC
IL-6	CCACTTCACAAGTCGGAGGCTTA	CCAGTTTGGTAGCATCCATCATTTC
L-10	GCCAGAGCCACATGCTCCTA	GATAAGGCTTGGCAACCCAAGTAA
TGF-β1	GTGTGGAGCAACATGTGGAACTCTA	CGCTGAATCGAAAGCCCTGTA
β-actin	CATCCGTAAAGACCTCTATGCCAAC	ATGGAGCCACCGATCCACA

iNOS, inducible nitric oxide synthase; CD, cluster of differentiation; Arg-1, arginase-1; TNF-α, tumor necrosis factor-α; IL, interleukin; TGF-β1, transforming growth factor-β1.

period of 95˚C for 30 sec, followed by a 2-step PCR program consisting of 40 cycles of 95˚C for 5 sec and 60˚C for 34 sec. All reactions were performed in duplicate. The following genes were analyzed (forward, reverse primers: Takara Bio Inc.): β-actin, inducible nitric oxide synthase (iNOS), CD86, arginase-1 (Arg-1), CD206, TNF-α, tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-10, and transforming growth factor-β1 (TGF-β1). The primers used in this experiment are listed in Table I.

Flow cytometry. LPMCs were washed with PBS and then blocked with anti-mouse CD16/32 Antibody (Clone:93, BioLegend) in a fluorescence-activated cell sorter (FACS) buffer for 10 min. After Fc receptor blockade, the LPMCs were stained with AF700-conjugated anti-CD11b (Clone: M1/70, BioLegend), PE-conjugated anti-c-MET (Clone: eBioclone7, Invitrogen; Thermo Fisher Scientific, Inc.), APC-A750-conjugated anti-CD86 (Clone: FA-11, Invitrogen; Thermo Fisher Scientific, Inc.), PC7-conjugated anti-iNOS (Clone: CXNFT, Invitrogen; Thermo Fisher Scientific, Inc.), eFluor-conjugated anti-Arg-1 (Clone: A1exF5, Invitrogen; Thermo Fisher Scientific, Inc.), and APC-conjugated anti-CD206 (Clone: C068C2, BioLegend) for 1 h on ice. Cells were washed and then resuspended in 4% paraformaldehyde PBS (Fujiwako), 2% bovine calf serum (Sigma-Aldrich; Merck KGaA), 0.1% sodium azide (Sigma-Aldrich; Merck KGaA), and 0.1% HEPES. They were then assayed on a CytoFLEX flow cytometer (Beckman Coulter) and analyzed using FlowJo (Becton Dickinson & Company).

Colon organ cultures. Segments of the distal colon were removed from each animal on day 5 after DSS administration, sectioned longitudinally, and washed in PBS containing penicillin and streptomycin. Segments of length 1 cm each were then placed in 24-well flat-bottom culture plates (Asahi Glass) containing 1 ml fresh RPMI-1640 medium supplemented with penicillin and streptomycin and incubated at 37˚C for 24 h. This experiment was performed on 13 mice from each group. The culture supernatants were stored at 30˚C until further analysis.

The concentrations of TNF-α, IL-6, IL-10, and TGF-β1 in the culture supernatants were measured using a Mouse Quantikine ELISA Kit (R&D Systems) according to the manufacturer's instructions and analyzed in duplicate using a microplate reader (Bio-Rad Laboratories) at 450 nm.

Statistical analysis. Differences between two or three groups were appropriately analyzed using Kruskal-Wallis test followed by Dunn test and Mann-Whitney U test (IBM SPSS version 28). P<0.05 indicated statistical significance. The *in vitro* experiments were performed at least twice independently.

Results

HGF-altered cytokine expression in LPMCs. First, we investigated whether LPMCs of mice with DSS-induced colitis expressed c-MET. The expression of *c-Met* of LPMC was higher in mice with DSS-induced colitis than in non-colitis mice. In addition, the percentage of c-MET-positive LPMCs was higher in mice with DSS-induced colitis than in non-colitis mice (Fig. 1A). Next, we investigated whether HGF affected the cytokine expression from LPMCs in mice with DSS-induced colitis or non-colitis. The LPMCs isolated from both mice groups were cultured under both conditions of presence and absence of HGF for 24 h. The expression of several cytokines and the genes encoding them was evaluated. A significant increase was observed in the expression of *IL-10, TGF-β, TNF-α,* and *IL-6* in LPMCs cultured with HGF compared to that in the untreated group (P=0.002, 0.004, 0.003, 0.005, respectively). Furthermore, the expression of IL-10 and IL-6 was numerically increased in cell culture supernatant with HGF-treated LPMCs compared to that in the untreated LPMCs (P=0.05), whereas no difference was observed in TNF-α and TGF-β1 expression in both groups (Fig. 1B and C).

HGF-induced polarization of M1 macrophages to M2-like phenotype. We investigated whether HGF affected the phenotype of macrophages, which are CD11b cells derived from the LPMCs of mice with DSS-induced colitis by MACS. No difference was observed in *iNOS* mRNA expression between

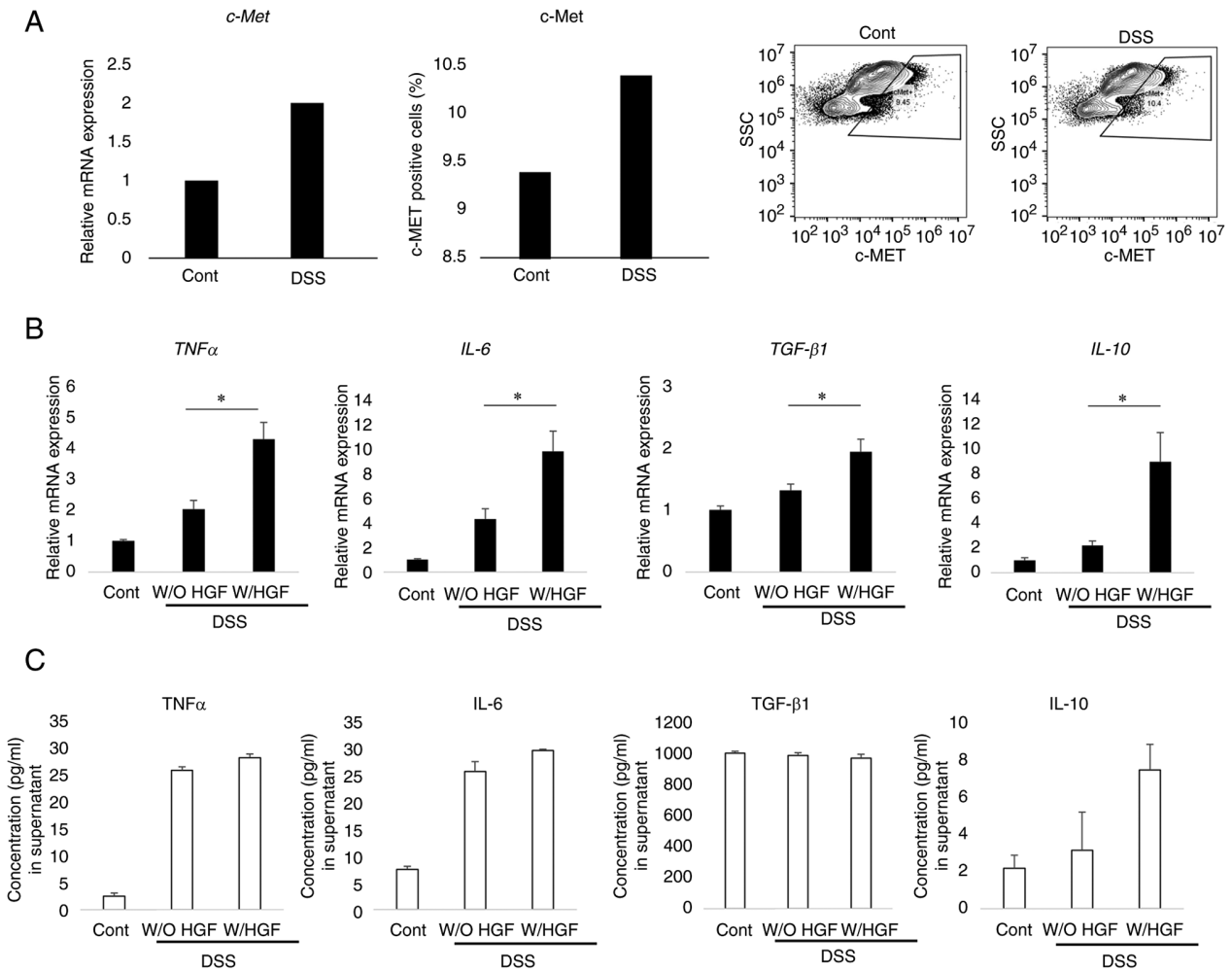

Figure 1. HGF upregulates the expression of c-MET and anti-inflammatory cytokines. (A) The LPMCs were isolated from mice with DSS-induced colitis or non-colitis mice. *c-MET* mRNA expression in LPMCs was analyzed using RT-qPCR. Obtained values were normalized to that of β-actin and expressed in comparison to that of the LPMCs isolated from untreated mice (n=3). The flow cytometry plots and the percentage of c-MET positive LPMCs from mice with DSS-induced and non-colitis mice were analyzed using flow cytometry (n=20). (B) *TNF-α*, *IL-6*, *TGF-β*, and *IL-10* mRNA expression in LPMCs treated with HGF or PBS for 24 h were analyzed using RT-qPCR. The obtained values were normalized to that of β-actin and expressed in comparison to that of the LPMCs isolated from untreated mice (n=10-16). (C) Concentrations of cytokines in the culture supernatant of LPMCs treated with HGF or PBS for 24 h. TNF-α, IL-6, TGF-β, and IL-10 expression was measured using ELISA (n=5). The results are shown as mean ± SD. Data were analyzed using (A) Mann-Whitney U test or (B and C) Kruskal-Wallis followed by Dunn test. *P<0.05. HGF, hepatocyte growth factor; c-MET, tyrosine-protein kinase Met; LPMCs, lamina propria mononuclear cells; DSS, dextran sodium sulfate; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; IL, interleukin; PBS, phosphate-buffered saline; TNF-α, tumor necrosis factor-α; Arg-1, arginase-1; TGF-β1, transforming growth factor-β1; ELISA, enzyme-linked immunosorbent assay.

HGF-treated and untreated CD11b cells, whereas *Arg-1* and *CD206* expression numerically increased in the CD11b cells treated with HGF compared to that in the untreated cells, and *CD86* expression decreased. The levels of M2 markers Arg-1 and CD206 increased in HGF-treated CD11b cells (Fig. 2A). Moreover, we analyzed M1 and M2 protein expression of CD11b cells treated with HGF using flow cytometry (Fig. 2B). In HGF-treated cells, iNOS and CD86-positive cell percentages were higher, whereas Arg-1 and CD206-positive cell percentages were lower than in untreated cells. These results suggest that HGF could induce transformation from the M1 phenotype to an M2-like phenotype in CD11b cells.

Furthermore, *IL-10* expression was significantly increased in CD11b cells treated with HGF compared to that in the untreated group (P=0.038), whereas *IL-6* expression decreased (P<0.001). We observed a significant increase in *TGF-β* expression in CD11b cells treated with HGF compared to that in the untreated group. However, no difference was observed in *TNF-α* expression between the HGF-treated and untreated CD11b cells. Moreover, the secretion of TNF-α was significantly increased in the cell culture supernatant of CD11b cells treated with HGF (P=0.029), and the secretion of IL-10 was numerically increased in the cell culture supernatant of CD11b cells treated with HGF, whereas the secretion of IL-6 was significantly decreased in the cells treated with HGF compared to that in the untreated cells (P=0.029). In addition, no difference was observed in TGF-β concentration between the two groups (Fig. 2C and D).

HGF ameliorate DSS-induced colitis. The DSS-induced colitis mouse model was evaluated on day 5 after DSS administration. The body weight of the mice in the vehicle group was significantly decreased compared to that in the HGF-treated group, and the DAI in the vehicle group was significantly increased compared to that in the HGF-treated group (P<0.05, Fig. 3A and B). Although no significant differences

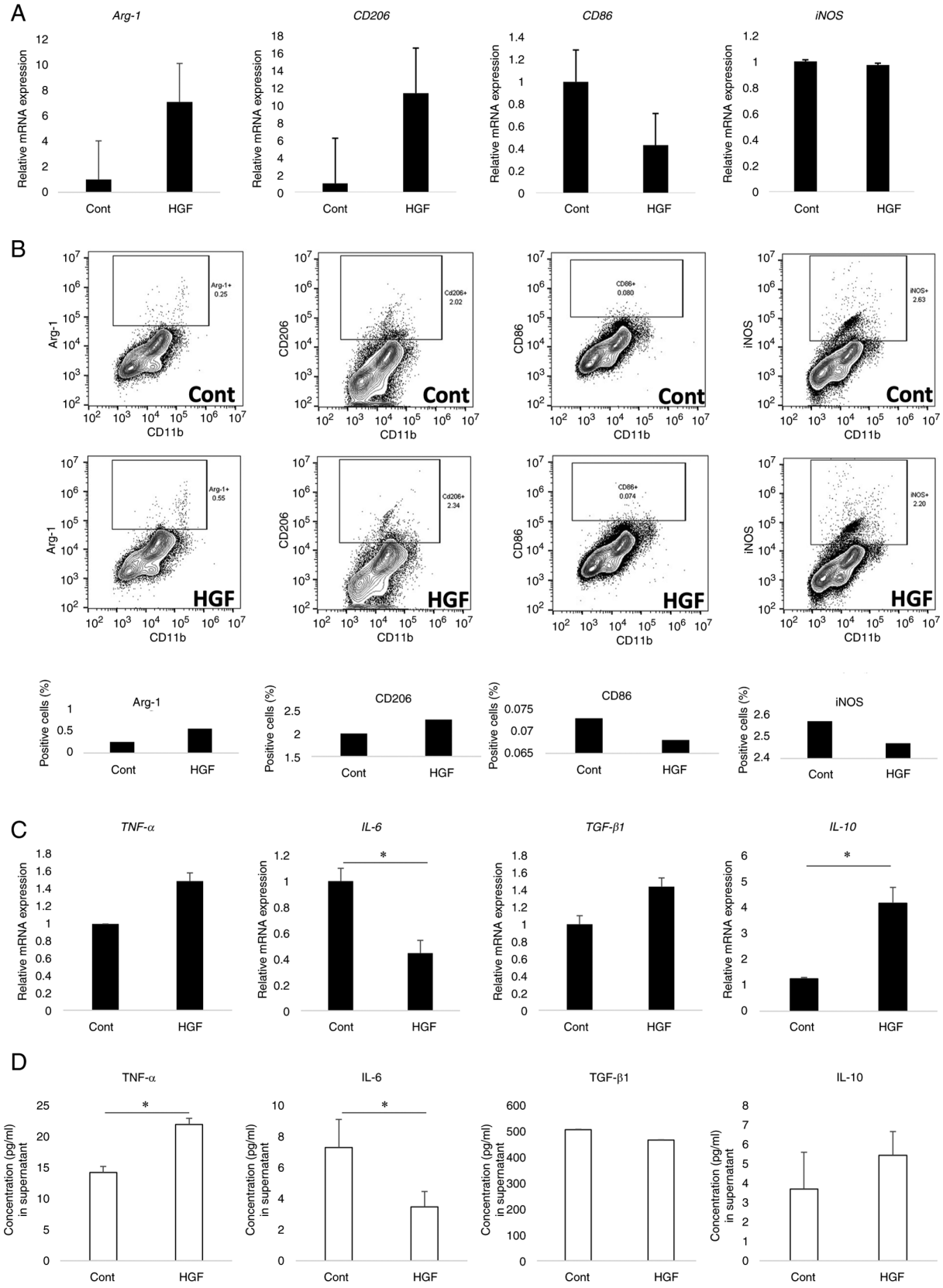

Figure 2. HGF shifts M1 phenotype to M2-like phenotype in macrophages. (A) Effects on expression of M1 and M2 markers by HGF treatment. iNOS and CD86 mRNA as M1 markers, Arg-1 and CD206 as M2 markers were analyzed using RT-qPCR. The values were normalized to that of β-actin and expressed in relation to CD11b-positive cells without HGF treatment. n=4. (B) Effects of HGF treatment on protein expression of M1 and M2 markers. iNOS, CD86, Arg-1, and CD206 were compared between HGF-treated and untreated CD11b-positive cells using flow cytometry. n=20. (C) Effects of HGF treatment on gene expression (n=8-11) and (D) secretion of cytokines (n=4) in CD11b-positive cells. TNF-α, IL-6, TGF-β, and IL-10 gene and protein expressions were measured using RT-qPCR and ELISA, respectively. The results are expressed as mean ± SD. Data were analyzed by Mann-Whitney U test. *P<0.05. HGF, hepatocyte growth factor; iNOS, inducible nitric oxide synthase; CD, cluster of differentiation; DSS, dextran sodium sulfate; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; IL, interleukin; TNF-α, tumor necrosis factor-α; Arg-1, arginase-1; TGF-β1, transforming growth factor-β1; ELISA, enzyme-linked immunosorbent assay.

were observed in colon length and pathological score between the vehicle and HGF-treated groups, the DAI and colon length in the HGF-treated group were lower and longer than those in the vehicle group, respectively (Fig. 3C-E).

Moreover, gene expression in CD11b cells separated from LPMCs of HGF-treated and vehicle groups was evaluated by RT-qPCR. *IL-6* expression was significantly decreased in the HGF-treated group compared to that in the vehicle group (P=0.029), whereas the expression levels of *TNF-α, IL-10,* and *TGF-β1* were not significantly different between the vehicle and HGF-treated groups (Fig. 3F). IL-6 secretion numerically decreased in the supernatant from the tissue culture of the HGF-treated group compared to that of the supernatant from the vehicle group; however, no significant difference was observed in cytokine secretion between the two groups (Fig. 3G).

Discussion

In this study, we demonstrated that HGF signaling induces the polarization of M1 to M2-like macrophages in the LPMCs of DSS-induced colitis mice. HGF-stimulated macrophages exhibited increased IL-10 production and decreased IL-6 production.

HGF, which was originally purified from the plasma of patients with fulminant hepatic failure, is the primary agent that promotes hepatocyte proliferation. HGF also functions as a pleiotropic factor, as it acts as a mitogen, morphogen, and motogen in multiple subsets of various epithelial cells (14,17). After HGF binds to the c-Met receptor, a signaling cascade is activated that increases several biological actions (proliferation/differentiation, survival, and motogenesis). HGF modulates a major agent that not only promotes the proliferation of hepatocytes but also of intestinal epithelial cells. Recombinant HGF and HGF gene therapy attenuates acute colitis, and the underlying mechanism could be the regeneration and repair of injured epithelial cells (16,25). In contrast, HGF also influences immune cells, such as macrophages, by altering macrophage polarization (26,27). Therefore, it is highly likely that the therapeutic effect of HGF uncovered in this study is not the promotion of epithelial regeneration but the therapeutic response resulting from its effect on immune cells (28-30).

Macrophages are one of the most abundant leukocytes in the intestinal mucosa and are essential for maintaining intestinal homeostasis. They are implicated in the pathogenesis of various disorders, such as IBD, offering the proteins on their surface that act as potential targets for novel therapies for IBD. The intestinal macrophage pool requires continual renewal from the circulating blood monocytes, unlike most other tissue macrophages, which appear to be derived from primitive precursors that subsequently self-renew (31). As many microbes always inhabit the intestinal tract, the macrophages must be regulated by some mechanism to prevent an excessive immune response against these organisms. However, an excessive immune response and abnormal differentiation of macrophages may be induced by intestinal microbes in IBD patients (32). The pro-inflammatory M1 (classically activated) macrophages produce cytokines, such as IL-6, IL-1, and TNFα; meanwhile, M2 (alternatively activated) macrophages produce IL-10 and TGFβ, which are thought to be associated with tissue repair. Therefore, the regulation of macrophage polarization is one of the most important mechanisms for maintaining immune homeostasis (33). In this study, the DSS-induced colitis mouse model was used as the IBD model. DSS-induced colitis occurs owing to the loss of epithelial barrier function and entry of intestinal microbes into the lamina propria. This results in an increase in the secretion of pro-inflammatory cytokines and chemokines, and M1 macrophages migrate into the lamina propria (34-36). c-MET is expressed by activated macrophages (11,12,26).

In addition, we reported that HGF induced a transformation from M1 to M2-like in macrophages extracted from bone marrow (26). M2 macrophages were generally negative for M1 markers (iNOS and CD86). However, the macrophages in our previous report primarily expressed M2 markers, but some M1 markers were expressed a little. Therefore, we defined the HGF-treated macrophages as M2-like. In this study, M1 macrophages also switched to M2-like macrophages, which showed low expression of an M1 marker (iNOS) and high expression of an M2 marker (Arg-1) after HGF treatment. This effect may be involved in the transition of macrophages from a pro-inflammatory to an anti-inflammatory phenotype during colonic inflammation attenuation and tissue repair.

The results of this study show that HGF increases the production of IL-6 by LPMC. IL-6 is produced by various cells, such as T cells, B cells, monocytes, and fibroblasts, and is generally known for its pro-inflammatory effects. LPMCs contain antigen-presenting cells, such as macrophages and dendritic cells, and many T cells and B cells. Therefore, cytokine expression in LPMCs would be affected by their cells. In contrast, HGF decreases IL-6 production by only activated macrophages (Fig. 2B), and IL-6 is known to promote the differentiation of pro-inflammatory Th17 cells while suppressing the production of $FoxP3^+$ regulatory T cells (37). Excessive IL-17 production by Th17 cells in the intestinal tract is involved in the development of IBD (38,39). In addition, IL-6 plays a pivotal role in angiogenesis, similar to vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β (40). Angiogenesis is essential in development and recovery from inflammation. HGF also suppresses IL-10 production by macrophages by stimulating M2-like macrophages (26). The function of IL-10 is important for regulating gut homeostasis during host defense, and IL-10 suppresses the activity of T-lymphocytes and mononuclear cells (41,42). The decreased expression of IL-6 and increased expression of IL-10 by the effect of HGF in this study affected T cells and improved intestinal inflammation.

This study had some limitations. The dose for this experiment was decided based on our previous experiment, specifically, intraperitoneal injections of HGF (0.1, 0.5, or 1.0 mg/kg) (43). These doses are sufficient for HGF to act; therefore, we administered a 10-fold-dose (10 mg/kg) in this experiment. However, we do not have dose-dependent data regarding HGF. Next, LPMCs and CD11b-positive cells showed some differences in cytokine secretion after HGF stimulation, which may be owing to the presence of LPMCs including T cells and dendritic cells. We were unable to examine the effects of HGF on immune cells other than

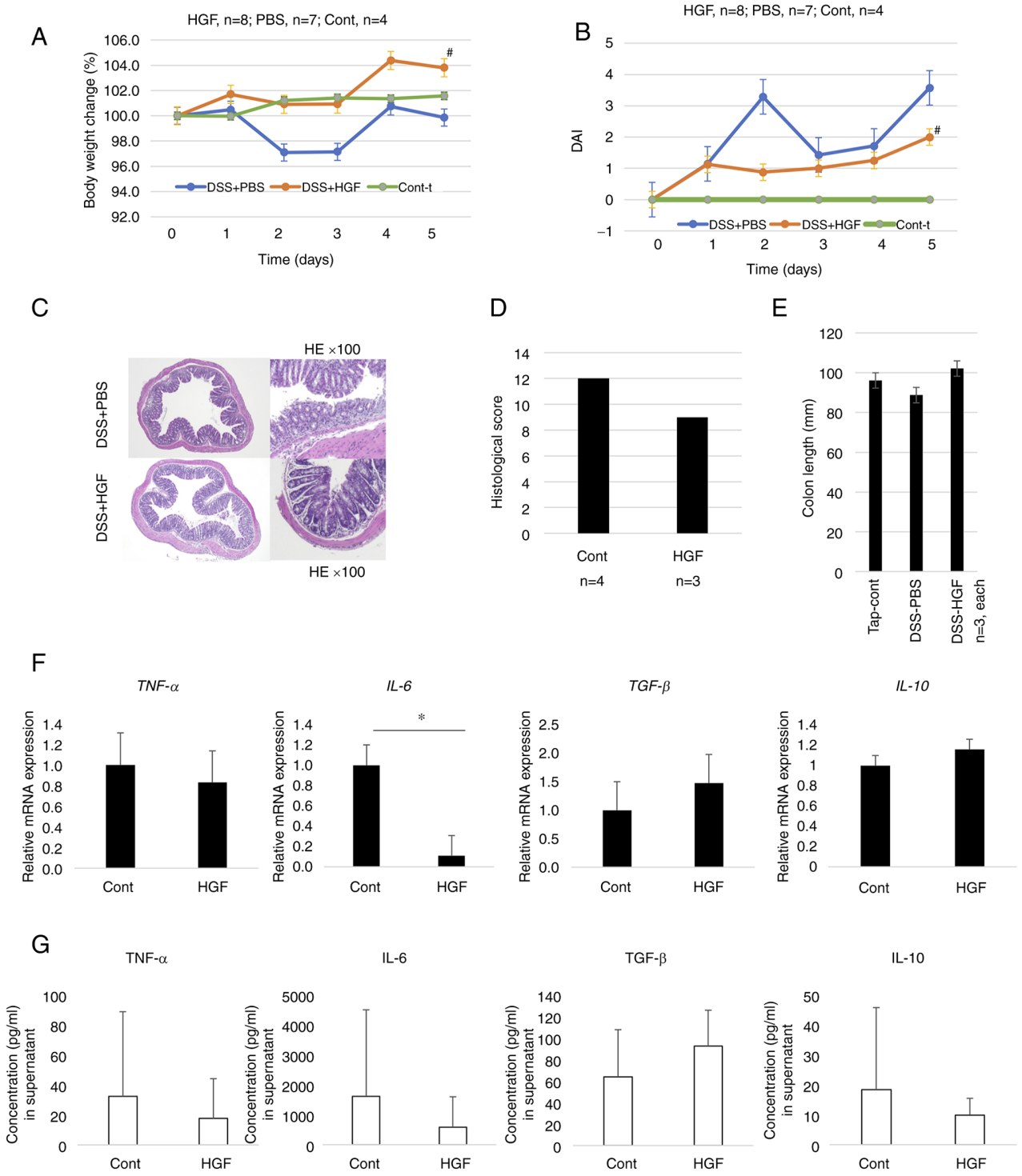

Figure 3. Effects of HGF in mice with DSS-induced colitis. (A) Final body weight relative to that on Day 0 (%). (B) Change in DAI scores. #P<0.05 (HGF vs. PBS group). (C) Histopathology of the distal large intestine. Magnification, x10, x100. colon tissue stained with hematoxylin and eosin (D) Histological score. (E) Colon lengths (mm). (F) Cytokine gene expression in CD11b-positive cells of colonic lamina propria mononuclear cells (n=8). (G) Expression of cytokines from the transverse colons obtained from PBS-treated and HGF-treated mice (n=6). Colons were cultured for 24 h in serum-free medium, and cytokine levels in the culture supernatants were measured using ELISA. Data were analyzed by (D, F and G) Mann-Whitney U test and (A, B and E) Kruskal-Wallis test followed by Dunn test *P<0.05. HGF, hepatocyte growth factor; DAI, disease activity index; DSS, dextran sulfate sodium; IL, interleukin; TNF-α, tumor necrosis factor-α; CD, cluster of differentiation; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay.

macrophages, and the effect of HGF on intestinal epithelial cells was not evaluated *in vivo*. We have also not been able to evaluate whether the use of MET inhibitors in this model counteracts the effects of HGF. Further analyses are required to clarify the effects of HGF on the immune system in the next experiment. In addition, we did not assess the downstream factors of HGF. Activation of MET by HGF induces the transphosphorylation of tyrosine kinase. Therefore, the downstream factors of HGF in intestinal immune cells will be investigated in the next study.

In conclusion, we have shown that a single round of intramuscular injection of adenoviral HGF is sufficient to inhibit apoptosis and reconstitute the epithelium in a mouse model of DSS-induced colitis. Based on these results, this approach shows promise as a clinical application of HGF in IBD treatment. HGF has the potential as an important new treatment modality for intestinal mucosal repair in IBD patients by mitigating intestinal inflammation, although additional preclinical biological studies are required.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YF performed the experiments, analyzed the data and wrote the manuscript. SK designed the study, analyzed the data and wrote the manuscript. YM, IK, NM, AT, HM and KK performed the experiments. ST and FS analyzed the data and revised the manuscript. AI designed the study and reviewed the manuscript. YF, YM and SK confirm the authenticity of all the raw data. All authors have contributed to the manuscript, and read and approved the final manuscript.

Ethics approval and consent to participate

The animal study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Kagoshima University.

Patient consent for publication

Not applicable.

Competing interests

AI received honoraria from Eisai Co. Ltd. YF, SK, YM, IK, NM, AT, HM, KK, FS and ST declare that they have no competing interests.

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