# Transgenic over-expression of macrophage migration inhibitory factor renders mice markedly more susceptible to experimental colitis

T. Ohkawara,*[†‡] K. Miyashita,*[†]
J. Nishihira,[‡] K. Mitsuyama,[¶]
H. Takeda,[†] M. Kato,[†] N. Kondo,[‡]
Y. Yamasaki,[§] M. Sata,[¶] T. Yoshiki,[‡]
T. Sugiyama** and M. Asaka[†]

[†]Department of Gastroenterology and Hematology,
Hokkaido University Graduate School of Medicine,
Sapporo, Japan, [‡]Genetic Lab Co. Ltd., Sapporo,
Japan, [§]Frontier Research Laboratories, Japan
Tabacco, Inc., Yokohama, Japan, [¶]Second
Department of Internal Medicine, Kurume
University School of Medicine, Kurume, Japan, and
**Third Department of Internal Medicine, Toyama
Medical and Pharmaceutical University, Toyama,
Japan

*The first and second authors equally contrib-
uted to this study.

## Summary

Enhanced production of macrophage migration inhibitory factor (MIF) is
recognized in patients with inflammatory bowel disease (IBD) and mice with
experimental colitis; however, the precise molecular function of MIF in colitis
is not fully understood. To further investigate this matter, we examined the
pathological features of MIF transgenic mice with dextran sulphate sodium
(DSS)-induced colitis. We generated transgenic mice carrying a murine MIF
cDNA driven by a cytomegalovirus enhancer and a β-actin/β-globin pro-
moter. Mice were orally administered 1–4% DSS in drinking water for 7 days.
Clinical disease activity, survival and histological features were evaluated. The
level of myeloperoxidase (MPO) activity in the colon tissue was measured to
assess neutrophil infiltration. The level of corticosterone in the serum was
measured by enzyme linked-immunosorbent assay (ELISA). MIF mRNA and
protein were markedly up-regulated in the colon and serum obtained from
MIF transgenic mice. The severity of the colitis induced by 1% DSS treatment
was markedly higher in MIF transgenic mice than in wild-type mice. We also
found that MPO activity was significantly higher in MIF transgenic mice than
wild-type mice in response to DSS stimulation. Interestingly, the corticoster-
one level remained unchanged in MIF transgenic mice. MIF enhances
DSS-induced colitis, in part via neutrophil accumulation and inhibition of
glucocorticoid bioactivity.

**Keywords:** corticosterone, dextran sulphate sodium-induced colitis, inflam-
matory bowel disease, macrophage migration inhibitory factor, neutrophil

## Introduction

Inflammatory bowel disease (IBD) is characterized as a
chronic and relapsing inflammation of the bowel, mainly
consisting of ulcerative colitis and Crohn's disease. Although
the aetiology of these intractable diseases remains unclear,
there are several reports suggesting the involvement of
proinflammatory cytokines in their pathogenesis and exac-
erbation [1–3]. Indeed, the neutralization of tumour necro-
sis factor (TNF)-α using anti-TNF-α antibody has been
found remarkably effective for the treatment of IBD [4,5].

Macrophage migration inhibitory factor (MIF) was orig-
inally identified as a cytokine-derived activated T lympho-
cyte *in vitro*; however, its precise biological function has
not sufficiently been elucidated until the cloning of MIF cDNA
[6]. MIF is released from the anterior pituitary gland in

response to endotoxic stimuli [7], and this response has been
shown to counter-regulate the immunosuppressive effects of
glucocorticoids [8,9]. Moreover, MIF is released from a vari-
ety of cells in response to stimuli as a proinflammatory
cytokine, and induces the production of TNF-α by mac-
rophages [10].

MIF plays an important role in the pathogenesis of
inflammatory diseases. The serum and the local expression
of MIF are significantly up-regulated in inflammatory dis-
eases such as rheumatoid arthritis [11], acute pulmonary
diseases [12] and glomerulonephritis [13]. It is of note that
neutralization of MIF activity by the anti-MIF antibody
ameliorated inflammation in these experimental models
[14–16]. It has been reported that MIF is increased in the
sera of patients with human IBD and experimental colitis
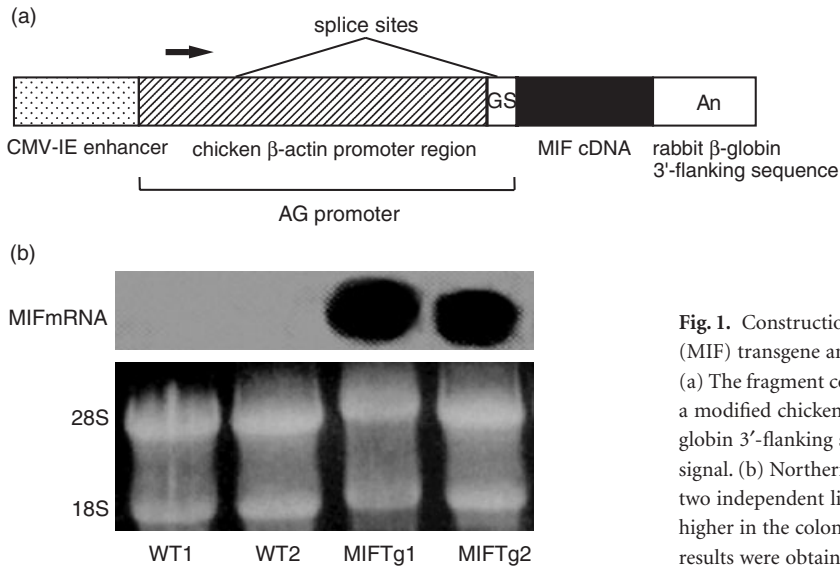[17–19]. In regard to immunohistochemistry, strong positive

(a)



(b)



Fig. 1. Construction of the macrophage migration inhibitory factor (MIF) transgene and expression of MIF mRNA in the colon of mice. (a) The fragment contained the cytomegalovirus (CMV)-IE enhancer, a modified chicken β-actin promoter, the MIF cDNA and a rabbit β-globin 3′-flanking sequence. GS, globin splice acceptor; An, poly A signal. (b) Northern blot analysis of MIF Tg and WT mouse colons in two independent lines. The levels of MIF mRNA expression were higher in the colon of MIF Tg mice than in that of WT mice. Similar results were obtained in three independent experiments.

staining of MIF has been observed in epithelial cells and infiltrating immune cells in colon tissue [18]. Moreover, neutralization of MIF bioactivity by the anti-MIF antibody significantly reduced the production of TNF-α, interferon (IFN)-γ and matrix metalloproteinases in the colon of mice. Although these results suggest that MIF plays a critical role in inflammation in the colon, the molecular basis of its mechanism of action has not been fully elucidated.

Dextran sulphate sodium (DSS)-induced colitis is considered to be a useful experimental model for investigating the mechanism of IBD because its pathological features are similar to those of the chronic inflammatory colonic disorders [20]. We have reported previously that MIF expression was up-regulated in the serum and colon tissues in murine DSS colitis and that anti-MIF antibody treatment effectively reduced the severity of colitis [18]. However, the effect of overexpression of endogenous MIF in the experimental model has not been fully clarified.

In this study, we generated MIF transgenic (Tg) mice to further clarify the role of MIF in the development of colitis, and investigated the effect of overexpression of MIF on DSS-induced colitis using MIF Tg mice.

## Materials and methods

### Mice and generation of MIF Tg mice

Eight- to 10-week-old C57BL6 mice weighing 22–25 g were purchased from Charles River Japan (Shizuoka, Japan). In this study, MIF Tg mice were established by using C57BL6 mice, which were in turn established from 8-week-old ICR mice (Charles River Japan, Shizuoka, Japan), as foster mothers [21]. In brief, the expression of the transgene was regulated by a hybrid promoter composed of a cytomegalovirus (CMV) enhancer and a β-actin/β-globin promoter [22]. The

cDNA sequence used for microinjection was obtained by polymerase chain reaction (PCR) amplification of a reverse transcript of mouse liver total RNA, which was cloned into pBluescript II KS (+), and the insert was confirmed by sequencing analysis. The cDNA was then subcloned into pCXN2, a derivative of pCAGGS [23,24]. The 2·7-kb SalI-HindIII fragment was separated from the vector sequence and used as a transgene. This fragment contained the CMV-IE enhancer, a modified chicken β-actin promoter, the MIF cDNA, and a rabbit β-globin 3′-flanking sequence (Fig. 1a). The transgene was microinjected into male pronuclei of fertilized C57BL6 mouse embryos, which were transplanted into the oviducts of pseudopregnant C57BL6 mice used as foster mothers. Founder mice, which had integrated the transgene, were bred with mice of the same strain. The resultant litters were analysed by Southern blotting of DNA extracted from their tails to distinguish between Tg mice and non-Tg mice. All mice were maintained on standard laboratory chow and given food and water *ad libitum*. This study adhered to the principles of the Declaration of Helsinki and was approved by the Animal Experiment Ethics Committee of the Graduate School of Medicine of Hokkaido University.

### Northern blot analysis

Colon tissues were obtained from mice under anaesthesia. Northern blot analysis was carried out as described previously [25]. In brief, RNA was isolated from these colon tissues using an Isogen RNA extraction kit (Wako, Osaka, Japan). These samples were separated into 10 μg units by electrophoresis on 1% agarose gel containing 0·6 M formaldehyde, and blotted onto nylon membrane filters. The probe for mouse MIF was labelled with [α-$^{32}$P]deoxycytidine triphosphate using a DNA random primer labelling kit (Takara, Kyoto, Japan). Hybridization was carried out at

42°C for 48 h. After hybridization the membranes were washed at 65°C for 15 min in ×0·2 standard saline citrate (SSC) with 0·1% sodium dodecyl sulphate (SDS). Bands were visualized by autoradiography on Kodak X-AR film. 18S and 28S of ribosomal RNA were used as a loading control.

### Induction and assessment of DSS colitis

Experimental colitis was induced by administration of 1·0% (wt/vol) DSS (molecular weight, approximately 40 000; ICN Biochemicals, Aurora, OH, USA) from day 0 to day 7 in drinking water. Mice were weighed and observed visually for rectal bleeding and diarrhoea every day beginning on day 0. The percentage weight change for each mouse was calculated compared to that on day 0. Prior to colon removal, mice were euthanized by intraperitoneal injection of thiopental. In a separate series of experiments, mice were sacrificed after various time intervals for measurement of cytokines and myeloperoxidase (MPO) activity. The colon was collected and stored at −80°C until use. The severity of colitis was evaluated by assessment of colon length and histological examination on day 7 after the first DSS administration. To examine the survival rate, the 1% DSS was switched to 4% DSS in drinking water, and the mice were inspected every day.

### Disease activity index based on clinical features

The disease activity index (DAI) was used to assess the grade of colitis based on a previously published scoring system [26] (Table 1). The score ranges from 0 to 4 (total score), and represents the sum of the scores for body weight loss, stool consistency and rectal bleeding divided by three. The DAI has been well correlated with tissue damage scores and with specific measurements of inflammation, such as myeloperoxidase (MPO) activity [26].

### Histological evaluation of colitis

The colon tissue samples were longitudinally opened, fixed with 10% neutral buffered formalin, embedded in paraffin,

**Table 1.** Scoring system for the disease activity index (DAI).

| Score | Weight loss (%) | Stool consistency | Occult/gross rectal bleeding |
|---|---|---|---|
| 0 | <1 | Normal | Negative |
| 1 | 1–5 | | |
| 2 | 5–10 | Loose stool | Haemo-occult positive |
| 3 | 10–20 | | |
| 4 | >20 | Diarrhoea | Gross bleeding |

These clinical criteria were used to evaluate the severity of colitis in mice. Scores were tailed for each category and then divided by three to obtain the DAI.

sectioned on glass slides, and stained with haematoxylin and eosin (H&E). For histological evaluation of the tissue damage, areas of inflammatory lesions were microscopically evaluated and scored by the pathologist in a blind fashion. The histological score was assessed as follows. The tissue damage grades were: 0, normal mucosa; 1, infiltration of inflammatory cells; 2, shortening of the crypt by less than half; 3, shortening of the crypt by more than half; 4, crypt loss; and 5, destruction of epithelial cells. The grades of the lesion extent were 0, 0%; 1, 1–20%; 2, 21–40%; 3, 41–61%; 4, 61–80%; and 5, 81–100%.

### Measurement of MPO activity in the colon

The level of tissue MPO was determined in accordance with a standard enzymatic procedure as described by Krawisz *et al.* [27], with minor modifications. In brief, the tissue specimen was homogenized in 50 mM potassium phosphate buffer (pH 6·0) with 0·5% hexadecyltrimethylammonium bromide using a Polytron-type homogenizer three times for 30 s each on ice. The sample was centrifuged at 20 000 *g* for 10 min at 4°C, and the supernatant was collected. This sample (100 μl) was added to 2·9 ml of 50 mM phosphate buffer (pH 6·0) containing 0·167 mg/ml O-dianisidine hydrocholoride and 0·0005% hydrogen peroxide. The absorbance at 460 nm in the sample was measured using a spectrometer at 25°C. The protein concentration of the supernatant was determined using a Bradford assay kit (Bio-Rad Laboratories, Hercules, CA, USA) for calibration, and the values were standardized using MPO purified from human leucocytes (Sigma, St Louis, MO, USA). One unit of change in MPO level was defined as the value that can degrade 1 μM $H_2O_2$ per minute at 25°C.

### Enzyme-linked immunosorbent assay for MIF and cytokines

MIF in the samples of plasma and colon tissue were measured with an enzyme-linked immunosorbent assay (ELISA) kit specific for MIF. This assay was performed in accordance with the procedure described previously [18].

### Enzyme-linked immunosorbent assay for corticosterone

Corticosterone in the serum was measured with an AngioMax Corticosterone ELISA kit (Angiopharm, O'Fallon, MO, USA). The assay was carried out according to the manufacturer's protocol.

### Statistics

All data are presented as the mean ± standard error (s.e.). The results were analysed statistically using analysis of variance (ANOVA) for ranks and post hoc tests using Student's unpaired *t*-test (StatView; SAS Institute, Cary, NC, USA).

**Table 2.** MIF contents in the serum and colon in C57B6 and macrophage migration inhibitory factor (MIF) transgenic (Tg) mice.

| | Serum (ng/ml) | Colon (ng/mg protein) |
|---|---|---|
| WT + water | $16\cdot4 \pm 1\cdot6$ | $37\cdot8 \pm 2\cdot7$ |
| WT + DSS | $25\cdot0 \pm 2\cdot8^*$ | $63\cdot0 \pm 8\cdot2^*$ |
| MIF Tg + water | $229\cdot8 \pm 20\cdot8^{**}$ | $1152\cdot0 \pm 115\cdot6^{**}$ |
| MIF Tg + DSS | $2016\cdot4 \pm 349\cdot4^{***}$ | $2053\cdot6 \pm 313\cdot6^{***}$ |

Values are expressed as the mean $\pm$ s.e. $n = 10$ mice per group. $^*P < 0\cdot05$ *versus* the values in untreated WT mice; $^{**}P < 0\cdot01$; $^{***}P < 0\cdot001$ *versus* the values in wild-type (WT) mice treated with 1% dextran sulphate sodium (DSS) for 7 days.

Survival curves of Kaplan–Meier plots were compared using a log rank test and final outcomes were calculated using Fisher's exact test. Values of $P < 0\cdot05$ were considered to indicate statistical significance.
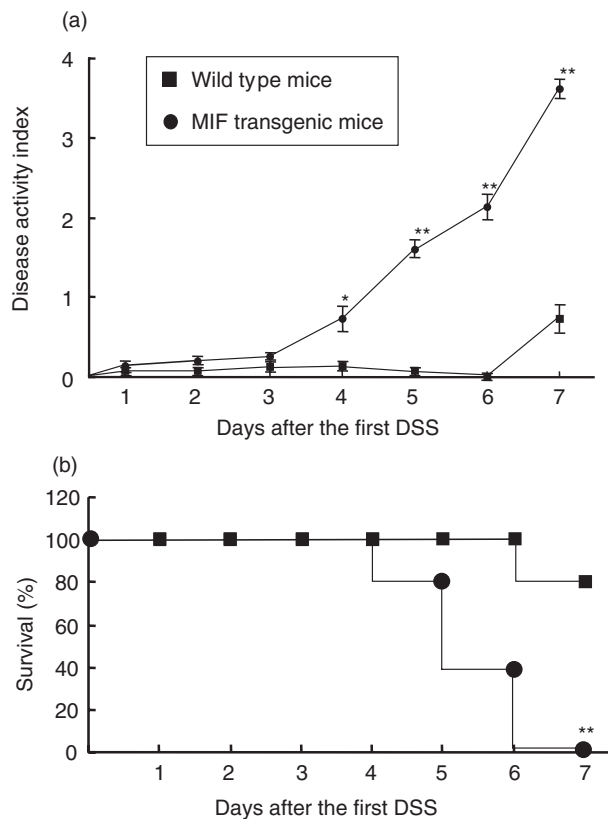


**Fig. 2.** (a) The disease activity index (DAI) in macrophage migration inhibitory factor (MIF) transgenic (Tg) mice was significantly higher than that in wild-type (WT) mice during the 1% dextran sulphate sodium (DSS) treatment. DAI was assessed daily during the 1% DSS treatment as described in Materials and methods. (b) Survival rate during DSS-induced colitis. Four per cent DSS in the drinking water was given to MIF Tg mice ($n = 10$) and WT mice ($n = 10$). Survival was recorded daily. All data are presented as the mean $\pm$ s.e. Statistical significance was assessed compared with WT mice on the same day. $^*P < 0\cdot05$, $^{**}P < 0\cdot001$.

## Results

### Enhancement of MIF mRNA and protein expression in the serum and colon tissues of MIF Tg mice

Northern blot analyses were performed on the colon samples from MIF Tg mice and those from untreated wild-type (WT) mice. The expression of MIF mRNA was stronger in MIF Tg mice than in WT mice (Fig. 1b). We determined the level of MIF in the colon tissues by ELISA. In MIF Tg mice, MIF levels in the serum and colon were more than 10-fold higher than in WT mice (Table 2). Moreover, the increase of MIF was remarkably higher in MIF Tg mice than in WT mice at day 7 after the induction of 1·0% DSS-induced colitis (Table 2).

### Severity of DSS-induced colitis in MIF Tg mice

The DAI value in WT mice increased on day 7 ($0\cdot73 \pm 0\cdot17$), and that in MIF Tg mice treated by 1% DSS was significantly higher than that in WT mice on days 4, 5, 6 and 7 ($0\cdot73 \pm 0\cdot15$, $1\cdot60 \pm 0\cdot11$, $2\cdot13 \pm 0\cdot16$ and $3\cdot60 \pm 0\cdot12$, respectively) (Fig. 2a). To examine the survival rate in DSS-induced colitis, mice were fed 4% DSS in drinking water. Eighty per cent of WT mice given 4% DSS survived until day 7. On the other hand, 20% of MIF Tg mice given 4% DSS died on day 4, and all had died by day 7 (Fig. 2b).

Measurement of colon length has often been used as a morphological parameter for the degree of inflammation in DSS colitis. To assess the colon length in the present study, mice in each group were sacrificed at the indicated times. At day 7 after DSS treatment, we found that the colon length in MIF Tg mice was significantly shorter than that in WT mice ($5\cdot4 \pm 0\cdot2$ and $6\cdot8 \pm 0\cdot1$, respectively) (Fig. 3).
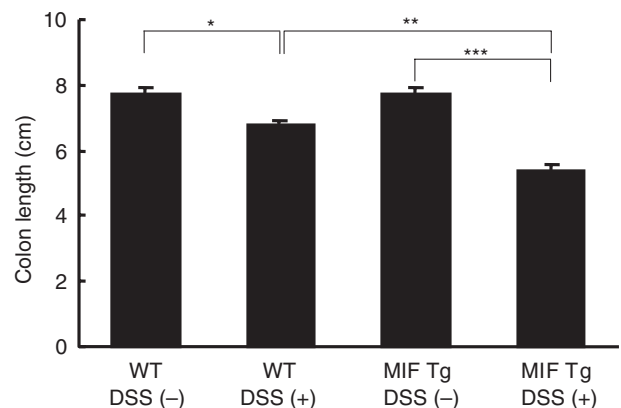


**Fig. 3.** Colon length at 7 days after 1% dextran sulphate sodium (DSS) treatment was significantly shorter in macrophage migration inhibitory factor (MIF) transgenic (Tg) mice than in wild-type (WT) mice. Data are expressed as the mean $\pm$ s.e. There were 10 mice in each group. Statistical significance was assessed compared with WT mice. $^*P < 0\cdot05$, $^{**}P < 0\cdot01$, $^{***}P < 0\cdot001$. WT, WT mice; Tg, Tg mice.
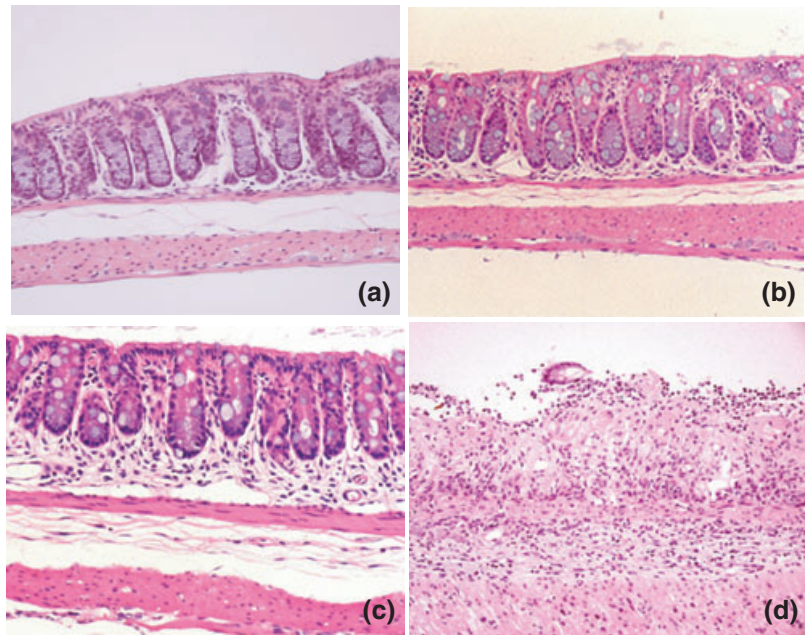
**Fig. 4.** Histology of colon in macrophage migration inhibitory factor (MIF) transgenic (Tg) mice. Sections of the colon from wild-type (WT) mice (a) and MIF Tg mice (b) without dextran sulphate sodium (DSS) treatment show normal crypts and no inflammatory infiltrate. (c) The section of WT mice 7 days after DSS treatment shows shortening of crypts and mild inflammatory cell infiltrate. (d) Section of a MIF Tg mouse at 7 days after DSS treatment shows complete crypt loss, destruction of the epithelial cells and severe inflammatory infiltrate. Magnification $\times 100$.

In the histological examination, both the colon samples of Tg mice and those of WT mice were normal (Fig. 4a,b). When mice were fed 1% DSS, the crypts in the mucosa of the colon in MIF Tg mice were shortened to less than half the length of those in WT mice at day 7 (Fig. 4c). The section of colon obtained from MIF Tg mice given 1% DSS for 7 days showed severe inflammatory cell infiltration and destruction of the epithelial cells in the mucosa (Fig. 4d). The scores for both tissue damage and the extent of the lesion were significantly higher in the colons of DSS-treated MIF Tg mice than in the colons of DSS-treated WT mice (Fig. 5).

### Increase of MPO activity in MIF Tg mice

MPO is an enzyme produced mainly by polymorphonuclear leucocytes and is well associated with the degree of neutrophil infiltration in tissues. The level of MPO activity was not significantly different between MIF Tg mice and WT mice before administration of DSS ($0 \cdot 2 \pm 0 \cdot 1$ and $0 \cdot 3 \pm 0 \cdot 1$, respectively). It was significantly higher in MIF Tg mice than in WT mice on days 3 and 7 after administration of DSS ($2 \cdot 0 \pm 0 \cdot 1$ *versus* $0 \cdot 6 \pm 0 \cdot 3$ ($P < 0 \cdot 05$) and $6 \cdot 6 \pm 0 \cdot 4$ *versus* $2 \cdot 2 \pm 0 \cdot 2$ ($P < 0 \cdot 01$), respectively) (Fig. 6).

### No increase of corticosterone level in the serum from MIF Tg mice with DSS-induced colitis

Corticosterone in mice is analogous to glucocorticoid in humans, and suppresses inflammation and the immune system in a similar manner. In this experiment, the level of corticosterone in the serum was measured by ELISA. The corticosterone level in serum was not significantly different between MIF Tg mice and WT mice prior to DSS treatment

($4 \cdot 1 \pm 0 \cdot 2$ and $5 \cdot 6 \pm 1 \cdot 0$, respectively) (Fig. 7). On the other hand, the corticosterone level in WT mice increased on day 7 after DSS treatment ($13 \cdot 0 \pm 3 \cdot 6$); however, the corticosterone level in MIF Tg mice remained unchanged ($4 \cdot 2 \pm 0 \cdot 5$) (Fig. 7).

## Discussion

In this study, we demonstrated for the first time that colitis could be induced by a very low concentration of DSS in MIF Tg mice. The histological features of inflammation and tissue damage in the colon of MIF Tg mice were remarkably severe compared with those of WT mice. In addition, MPO activity was markedly higher in MIF Tg mice than WT mice, and this increase in MPO activity occurred at a very early stage after DSS treatment. The level of corticosterone significantly increased in the serum from WT mice given DSS, whereas this level showed only minimal change in MIF Tg mice.

It has been suggested that macrophages play a critical in the induction of DSS-induced colitis. Dieleman *et al.* [28] demonstrated that acute DSS-induced colitis occurred in severe combined immunodeficient (SCID) mice deficient of T and B lymphocytes. Although the histological features of colitis in SCID mice are similar to those of DSS-induced colitis in BALB/c mice, the cells that had infiltrated into the lesions of the SCID mice consisted of mainly macrophages, along with a smaller number of lymphocytes and granulocytes. Okayasu *et al.* [20] demonstrated that the phagocytes in the mucosa of mice with DSS-induced colitis were mainly macrophages. Similarly, we found that large numbers of phagocytes had infiltrated into the mucosa of mice with DSS-induced colitis in the present study. Onodera *et al.* [29]
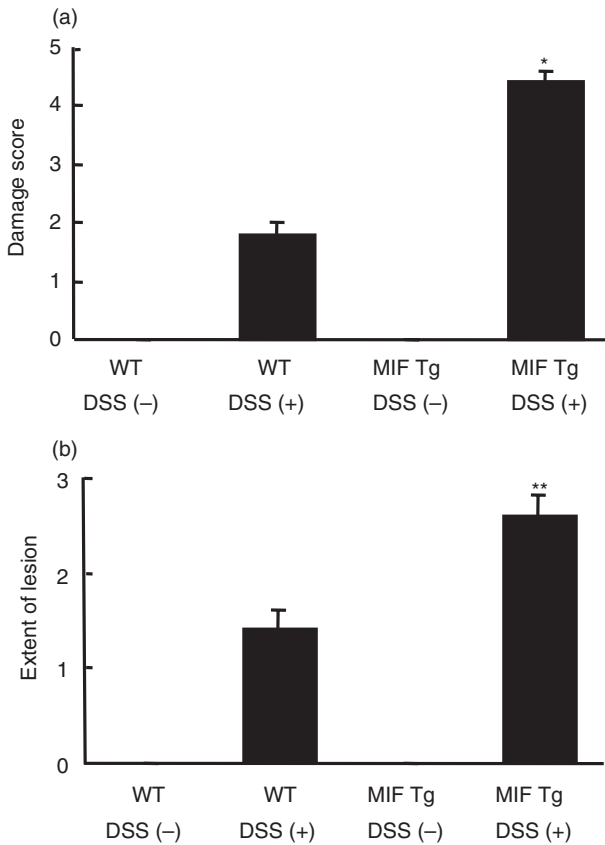
(a)



(b)



**Fig. 5.** The histological scores for the colons of macrophage migration inhibitory factor (MIF) transgenic (Tg) mice ($n = 7$) at day 7 after administration of 1·0% dextran sulphate sodium (DSS) were significantly higher than those of wild-type (WT) mice ($n = 7$). Histological evaluation of the tissue damage (a) and the extent of inflammatory lesions (b) was made using a microscope and quantified by the method described in Materials and methods. Statistical significance was assessed compared with WT mice. *$P < 0.05$, **$P < 0.001$.

reported that MIF could modulate the function of phagocytosis in macrophages. They demonstrated that recombinant MIF enhanced the phagocytosis of foreign particles in the RAW 264·7 cells in a dose-dependent manner. In this study, we showed that DSS-induced colitis in MIF Tg mice was more severe than that in WT mice. This result suggests that MIF has the potential to enhance the phagocytosis of DSS by macrophages in the colon.

Makita *et al.* [15] reported that MIF modulated neutrophil accumulation in the lungs in an acute pneumonitis rat model. Further, they showed that injection of lipopolysaccharides (LPS) up-regulated MIF expression in both the serum and the lungs. In their model, neutrophil accumulation increased significantly in the lesions of alveoli. Neutralization of MIF by an anti-MIF antibody was effective therapeutically and reduced the alveolar neutrophil accumulation. Baumann *et al.* [30] reported that MIF played a critical role in the apoptosis of neutrophils *in vitro*. MIF delayed the apoptosis of cultured neutrophils obtained
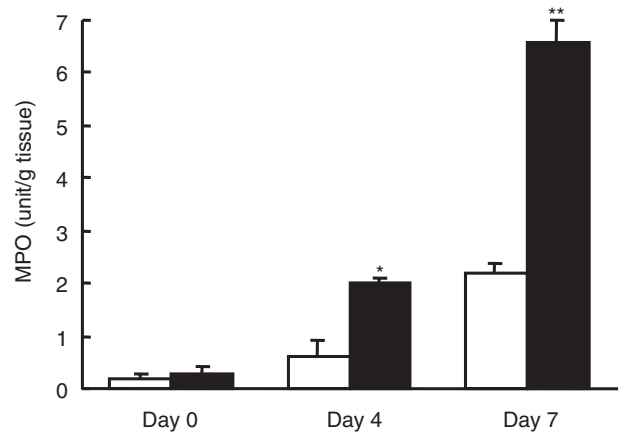


**Fig. 6.** The myeloperoxidase (MPO) level in the colon of macrophage migration inhibitory factor (MIF) transgenic (Tg) mice ($n = 6$) increased at days 3 and 7 after treatment of 1% dextran sulphate sodium (DSS) compared with that of wild-type (WT) mice ($n = 6$). MPO level was determined as described in Materials and methods. Open box, WT mice; closed box, MIF Tg mice. All data are expressed as the mean ± s.e. Statistical significance was assessed compared with WT mice on the same day. *$P < 0.05$, **$P < 0.01$.
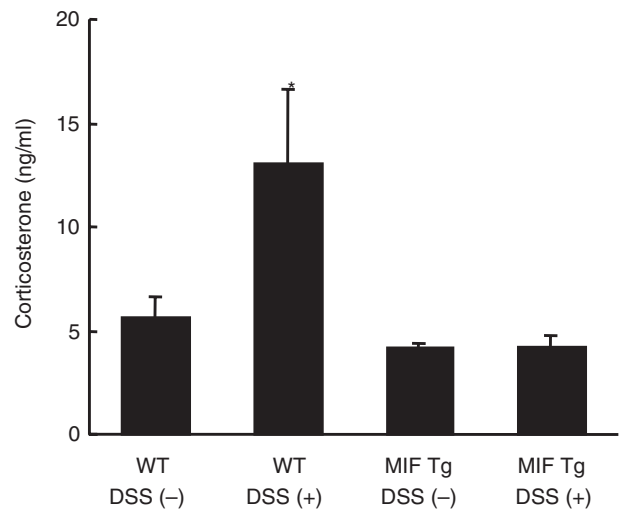


**Fig. 7.** Corticosterone level in the serum from macrophage migration inhibitory factor (MIF) transgenic (Tg) mice and wild-type (WT) mice. The serum obtained before and 7 days after 1% dextran sulphate sodium (DSS) treatment was measured by enzyme-linked immunosorbent assay (ELISA). Corticosterone was significantly increased in WT mice treated with DSS compared with non-treated WT, non-treated MIF Tg and DSS-treated MIF Tg mice (*$P < 0.05$). The level of corticosterone in MIF Tg mice did not change between before and 7 days after DSS treatment. No significant differences were observed between non-treated WT, non-treated MIF Tg and DSS treated MIF Tg mice. All data are expressed as the mean ± s.e. There were five mice in each group. WT, WT mice; Tg, Tg mice.

from the abdominal cavity in mice in a time- and dose-dependent manner, whereas the anti-MIF antibody promoted programmed cell death. This fact suggests that MIF expression contributes to the recruitment of neutrophils at inflammatory loci and promotes acute inflammation. As for MIF Tg mice, we found increased neutrophil accumulation and rapid increase of the activity of MPO, a molecular component of neutrophils, on day 3 after the first DSS treatment. On day 7 after DSS treatment, MPO activity was significantly higher in MIF Tg mice than in WT mice. Consistent with these findings, de Yong et al. [17] reported that chronic colitis induced by transfer of T lymphocytes containing high levels of CD45RB occurred in Rag-2-deficient mice and MIF was produced by innate immune cells.

Sasaki et al. [21] observed podocyte injury and progressive mesangial sclerosis in the kidneys of MIF Tg mice. In their study, MIF was expressed strongly in the mesangial cells and phagocytes of the MIF Tg mice. However, infiltration of macrophages into the kidneys of MIF Tg mice was not significantly greater than that in WT mice. We found here that there were no differences between MIF Tg and WT mice with regard to histological findings of the colon under normal conditions. However, low-dose DSS, which induced only minimal colitis in WT mice, induced severe colitis in MIF Tg mice. The levels of MIF protein in both the serum and colon tissues were significantly increased in MIF Tg mice compared with those in WT mice. Moreover, DSS treatment increased the MIF level in the serum and colon tissue of MIF Tg mice. These results indicate that the increased MIF content produced by the MIF gene transfer contributes to the inflammatory process in DSS-induced colitis.

Corticosterone in mice is analogous to glucocorticoid in humans. Glucocorticoids show critical anti-inflammatory and pro-apoptotic activities within the immune system. The anti-inflammatory action of glucocorticoids originates in the suppression of the expression of proinflammatory genes at the level of DNA transcription. Calandra et al. [8] demonstrated that MIF modulated the secretion and activity of glucocorticoids in vitro. A low concentration of glucocorticoids induced MIF production from macrophages. Moreover, MIF over-rode glucocorticoid-mediated inhibition of cytokine secretion by LPS-stimulated monocytes and overcame glucocorticoid protection against lethal endotoxaemia. Another study [31] reported that exogenous glucocorticoids modulated the expression of MIF in rat tissues after ablation of the hypothalamic-pituitary-adrenal axis. Interestingly, we demonstrated that overexpression of MIF inhibited the level of corticosterone in vivo. The level of corticosterone in the serum from WT mice given DSS for 7 days increased significantly. On the other hand, the level of corticosterone in the serum remained unchanged in MIF Tg mice given DSS. This fact suggests that overexpression of MIF suppresses the up-regulation of corticosterone and progresses the inflammatory process in DSS-induced colitis.

In conclusion, we have shown for the first time that transgene of MIF accelerates inflammation in murine experimental colitis. DSS-induced colitis in MIF Tg mice was clinically and histologically more severe than that in WT mice. MPO activity, which is one of the inflammatory parameters in acute colitis, was remarkably increased in the MIF Tg mice. Moreover, overexpression of MIF inhibited the glucocorticoid response in MIF Tg mice. Our results suggest that MIF is critical for the inflammatory process in DSS-induced colitis. Suppression of MIF bioactivity may have therapeutic implications for the treatment of IBD.

## Acknowledgements

## References

1 Podolsky DK. Inflammatory bowel disease. N Engl J Med 2002; **347**:417–29.

2 Sartor RB. Pathogenesis and immune mechanisms of chronic inflammatory bowel diseases. Am J Gastroenterol 1997; **92**:S5–11.

3 Papadakis KA, Targan SR. Role of cytokines in the pathogenesis of inflammatory bowel disease. Annu Rev Med 2000; **51**:289–98.

4 Targan SR, Hanauer SB, van Deventer SJ et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. N Engl J Med 1997; **337**:1029–35.

5 van Dullemen HM, van Deventer SJ, Hommes DW et al. Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). Gastroenterology 1995; **109**:129–35.

6 Weiser WY, Temple PA, Witec-Giannoti JS et al. Molecular cloning of a cDNA encoding a human macrophage migration inhibitory factor. Proc Natl Acad Sci USA 1989; **86**:7522–7.

7 Bernhagen J, Calandra T, Mitchell RA et al. MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia. Nature 1993; **365**:756–9.

8 Bucala R. MIF rediscovered: pituitary hormone and glucocorticoid-induced regulator of cytokine production. FASEB J 1996; **7**:19–24.

9 Calandra T, Bernhagen J, Metz CN et al. MIF as a glucocorticoid-induced modulator of cytokine production. Nature 1995; **377**:68–71.

10 Calandra T, Bernhagen J, Mitchell RA et al. The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. J Exp Med 1994; **179**:1895–902.

11 Onodera S, Tanji H, Suzuki K, Kaneda K et al. High expression of macrophage migration inhibitory factor in the synovial tissues of rheumatoid joints. Cytokine 1999; **11**:163–7.

12 Donnelly SC, Haslett C, Reid PT et al. Regulatory role for macrophage migration inhibitory factor in acute respiratory distress syndrome. Nat Med 1997; **3**:320–3.

13 Lan HY, Yang N, Nikolic-Paterson DJ et al. Expression of macrophage migration inhibitory factor in human glomerulonephritis. Kidney Int 2000; **57**:499–509.

14 Mikulowska A, Metz CN, Bucala R *et al.* Macrophage migration inhibitory factor is involved in the pathogenesis of collagen type II-induced arthritis in mice. J Immunol 1997; **158**:5514–17.

15 Makita H, Nishimura M, Miyamoto K *et al.* Effect of anti-macrophage migration inhibitory factor antibody on lipopolysaccharade-induced pulmonary neutrophil accumulation. Am J Respir Crit Care Med 1998; **158**:573–9.

16 Lan HY, Bacher M, Yang N *et al.* The pathogenic role of macrophage migration inhibitory factor in immunologically induced kidney disease in the rat. J Exp Med 1997; **185**:1455–65.

17 de Jong YP, Abadia-Molina AC, Satoskar AR *et al.* Development of chronic colitis is dependent on the cytokine MIF. Nat Immunol 2001; **2**:1061–6.

18 Ohkawara T, Nishihira J, Takeda H *et al.* Amelioration of dextran sulfate sodium-induced colitis by anti-macrophage migration inhibitory factor antibody in mice. Gastroenterology 2002; **123**:256–70.

19 Murakami H, Akbar SMF, Matsui H *et al.* Macrophage migration inhibitory factor in the sera and at the colonic mucosa in patients with ulcerative colitis: clinical implications and pathogenic significance. Eur J Clin Invest 2001; **31**:337–43.

20 Okayasu I, Hatakeyama S, Yamada M *et al.* A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology 1990; **98**:694–702.

21 Sasaki S, Nishihira J, Ishibashi T *et al.* Transgene of MIF induces podocyte injury and progressive mesangial sclerosis in the mouse kidney. Kidney Int 2004; **65**:469–81.

22 Ayagi Y, Isaka Y, Akagi A *et al.* Transcriptional activation of a hybrid promoter composed of cytomegalovirus enhancer and β-actin/β-globin gene in glomerular epithelial cells *in vivo*. Kidney Int 1997; **51**:1265–9.

23 Miyazaki J, Takaki S, Araki K *et al.* Expression vector system based on the chicken beta-actin promoter directs efficient production of interleukin-5. Gene 1989; **79**:269–77.

24 Niwa H, Yamamura K, Miyazaki J. Efficient selection for high-expression transfectants with a novel eukaryotic vector. Gene 1991; **108**:193–9.

25 Nishihira J, Koyama Y, Mizue Y. Identification of macrophage migration inhibitory factor (MIF) in human vascular endothelial cells and its induction by lipopolysaccharide. Cytokine 1998; **10**:199–205.

26 Cooper HS, Murthy SNS, Shah RS *et al.* Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest 1993; **69**:238–50.

27 Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. Gastroenterology 1984; **87**:1344–50.

28 Dieleman LA, Ridwan BU, Tennyson GS *et al.* Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. Gastroenterology 1994; **107**:1643–52.

29 Onodera S, Suzuki K, Matsuno T *et al.* Macrophage migration inhibitory factor induces phagocytosis of foreign particles by macrophages in autocrine and paracrine fashion. Immunology 1997; **92**:131–7.

30 Baumann R, Casaulta C, Simon D *et al.* Macrophage migration inhibitory factor delays apoptosis in neutrophils by inhibiting the mitochondria-dependent death pathway. FASEB J 2003; **17**:2221–30.

31 Fingerle-Rowson G, Koch P, Bikoff R *et al.* Regulation of macrophage migration inhibitory factor expression by glucocorticoids *in vivo*. Am J Pathol 2003; **162**:47–5.