

Amelioration of Murine Experimental Colitis by Inhibition of Mucosal Addressin Cell Adhesion Molecule-1¹

SHINGO KATO, RYOTA HOKARI, KOJI MATSUZAKI, ATSUHIRO IWAI, ATSUSHI KAWAGUCHI, SHIGEAKI NAGAO, TORU MIYAHARA, KAZURO ITOH, HIROMASA ISHII, and SOICHIRO MIURA

Second Department of Internal Medicine, National Defense Medical College, Saitama, Japan (S.K., R.H., K.M., A.I., A.K., S.N., T.M., K.I., S.M.); and Department of Internal Medicine, Keio University, School of Medicine, Tokyo, Japan (H.I.)

Accepted for publication May 11, 2000 This paper is available online at <http://www.jpet.org>

ABSTRACT

Mucosal addressin cell adhesion molecule-1 (MAdCAM-1) is an adhesion molecule that mediates recruitment of lymphocytes into the gut mucosa. Attenuation of excessive expression of MAdCAM-1 in the inflamed mucosa could be useful for treatment of inflammatory bowel diseases. The aim of this study was to investigate whether anti-MAdCAM-1 antibody has a prophylactic effect on experimental colitis induced by dextran sulfate sodium (DSS). Colitis was induced by orally feeding BALB/c mice 5% DSS (mol. wt. 5000). Mice were sacrificed at intervals up to 21 days after administration to evaluate the changes over time in intestinal damage. The infiltrating lymphocytes and their subpopulations, and the expression of cell adhesion molecules were determined by immunohistochemistry. In another set of experiments, the attenuating effect of i.p.-injected anti-MAdCAM-1 antibody on colonic lesions was

evaluated on day 14. Significant histological damage with shortening of crypts was observed on day 14 in colonic mucosa of DSS-treated mice. Before mucosal inflammation had become significant, expression of MAdCAM-1 was already increased in the microvessels of lamina propria on day 7. Significant infiltration of $\beta 7$ -integrin-positive T and B cells in the mucosa was then noted on day 14. Administration of anti-MAdCAM-1 antibody significantly reduced colonic injury as well as the infiltration of $\beta 7$ -integrin-positive lymphocytes in the colonic mucosa. This antibody also was effective when given 7 days after the start of DSS treatment. In the present study, we demonstrated that anti-MAdCAM-1 antibody significantly ameliorates DSS-induced colitis, suggesting that MAdCAM-1 may be useful for control of inflammatory bowel diseases.

Lymphocytes always recirculate from blood to lymphoid tissue to maintain immunological surveillance. Lymphocyte homing from blood to lymphoid tissue and inflammatory sites depends on the interaction between lymphocytes and high endothelial venules. This interaction is in multistep theory mediated by selectins, integrins, and Ig superfamily adhesion molecules (Butcher, 1991; Shimizu et al., 1992; Springer, 1994; Bargatze et al., 1995). The homing of lymphocytes appears to be organ specific, and lymphocyte homing to Peyer's patches and mucosal sites is thought to be regulated by $\alpha 4\beta 7$ integrin and its counter ligand mucosal addressin cell adhesion molecule-1 (MAdCAM-1) (Berlin et al., 1993). MAdCAM-1 is an Ig superfamily adhesion molecule expressed on mucosal endothelium, and blocking monoclonal antibodies for $\alpha 4\beta 7$ integrin and MAdCAM-1 have been reported to inhibit normal homing of $\alpha 4\beta 7$ -positive lym-

phocytes to the small intestine, Peyer's patches, and mesenteric lymph nodes (Hamann et al., 1994).

It is becoming increasingly apparent that inflammation of the intestine is associated with enhanced expression of adhesion molecules in both experimental animals and humans (Nakamura et al., 1993; Miura et al., 1996). In human ulcerative colitis and Crohn's disease, the expression of MAdCAM-1 is up-regulated in factor VIII-positive vessels in inflamed colonic mucosa (Briskin et al., 1997). It has been reported that the expression of MAdCAM-1 is increased in some animal models of colitis (Viney et al., 1996; McDonald et al., 1997; Picarella et al., 1997; Connor et al., 1999), in islet vessels of nonobese diabetic mice (Hanninen et al., 1993; Faveeuw et al., 1994), and in spinal cord vessels of experimental allergic encephalomyelitis (O'Neill et al., 1991). However, whether enhanced expression of MAdCAM-1 in inflamed intestinal mucosa plays a significant role in the development of inflammatory bowel diseases has not been clearly determined.

Oral administration of dextran sulfate sodium (DSS) in-

Received for publication February 28, 2000.

¹This study was supported in part by grants from the National Defense Medical College of Japan and by Funds for Food Allergy from the Ministry of Welfare of Japan.

ABBREVIATIONS: MAdCAM-1, mucosal addressin cell adhesion molecule-1; DSS, dextran sulfate sodium; mAb, monoclonal antibody; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; FITC, fluorescein isothiocyanate; PMNL, polymorphonuclear leukocyte; IL, interleukin; TNF, tumor necrosis factor.

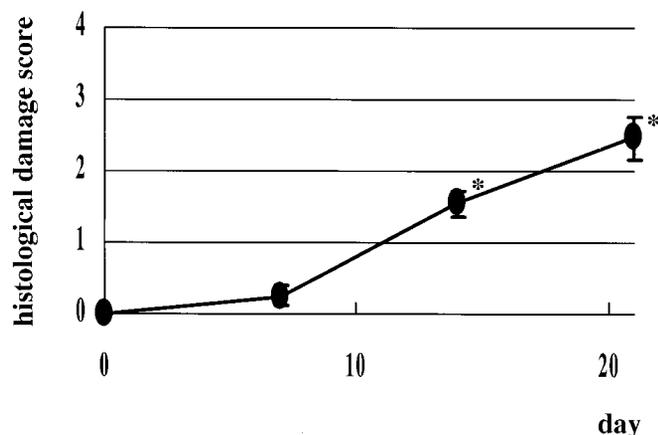


Fig. 1. Changes over time in histological damage score of distal colon in DSS-treated mice. BALB/c mice were fed orally 5% DSS (mol. wt. 5000) in drinking water. Mice were sacrificed on day 0, 7, 14, and 21. The tissue sections examined under a light microscope were given a score on a 0 to 4 scale based on the criteria as described under *Materials and Methods*. Results are expressed as the mean \pm S.E. of six animals. * $P < .05$ compared with day 0 (controls).

duces acute and chronic colitis in mice (Okayasu et al., 1990; Cooper et al., 1993). The histology of this experimental colitis includes destruction of crypts and infiltration of inflammatory cells, including lymphocytes, macrophages, and granulocytes in the inflamed colon. This model resembles human ulcerative colitis, and has been widely investigated as a form of reproducible mucosal colonic inflammation in mice (Elson et al., 1995). However, whether MAdCAM-1 plays a role in the pathogenesis of DSS-induced colitis remains unknown. The aims of this study were 1) to evaluate the time course changes in expression of MAdCAM-1 and inflammatory cell infiltration in the colonic mucosa of DSS-induced colitis, and 2) to examine whether treatment with anti-MAdCAM-1 antibody has a prophylactic effect on the development of this model.

Materials and Methods

Induction of Colitis. Specific pathogen-free female BALB/c mice (18–20 g, 8 weeks old) were housed in wire-mesh-bottom cages in our animal laboratory center. The care and use of laboratory animals were in accordance with the National Institutes of Health guidelines. DSS (mol. wt. 5000; Wako, Osaka, Japan) was dissolved in drinking water to a final concentration of 5%. All mice were allowed free access to animal chow (CE-2; Nippon Clea Inc., Osaka, Japan) and water. Occult blood in the feces was evaluated by the orthotolidine and the guajac methods, and blood clot around the anus was considered as the gross blood per rectum.

Assessment of Histological Damage. Changes over time in colonic damage induced by DSS were determined. To estimate the formation of colitis, mice were sacrificed on days 0, 7, 14, and 21 after the start of induction of colitis. Under pentobarbital anesthesia, the colon was removed and opened longitudinally. The colon was divided into three segments (cecum, proximal and distal colon). Each segment was cut in half longitudinally and one of each segment was fixed in 10% buffered formalin. Tissues were embedded in paraffin and 4- μ m longitudinal sections were stained with H&E. Histological damage score was measured by the method of crypt scoring by Cooper et al. (1993): grade 0, intact crypt; grade 1, loss of the basal one-third of the crypt; grade 2, loss of the basal two-thirds of the crypt; grade 3, loss of entire crypt with the surface epithelium remaining intact; and grade 4, loss of both the entire crypt and surface epithelium. Histological damage score was measured in each seg-

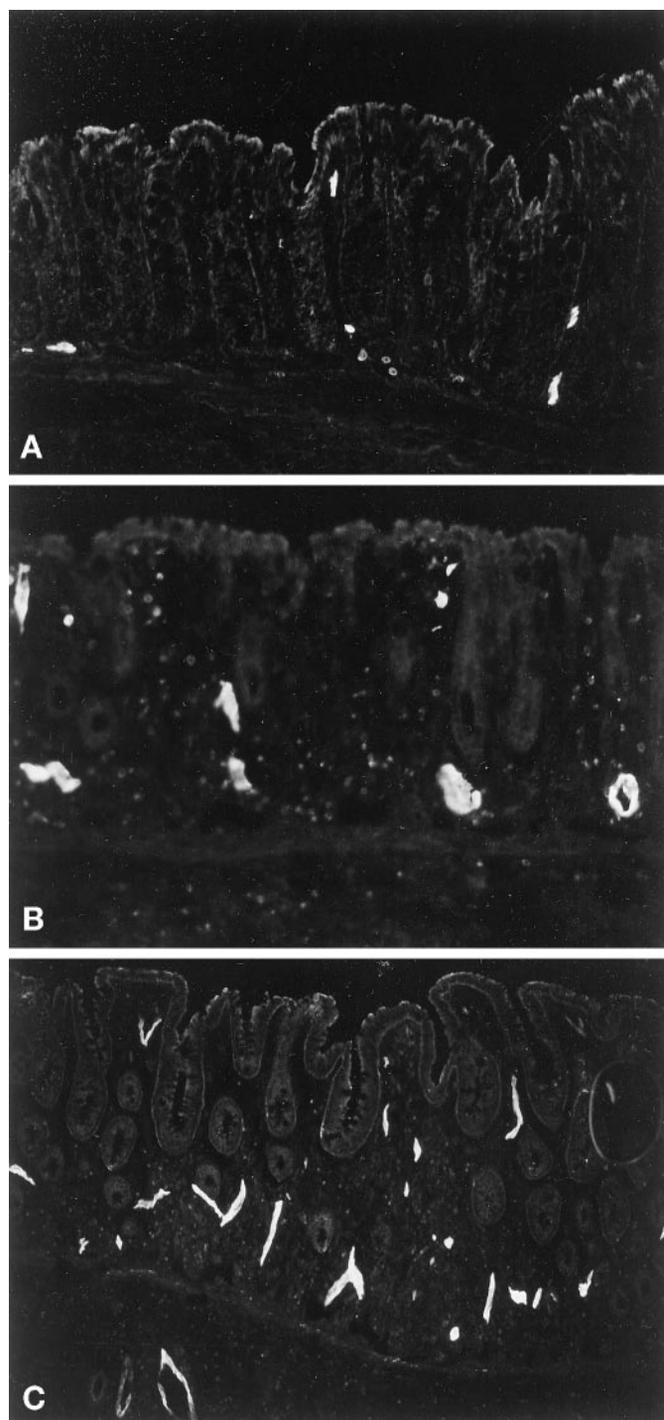


Fig. 2. Immunohistochemical study of MAdCAM-1 expression in the colonic mucosa of DSS-treated mice. A, MAdCAM-1 expression of non-treated mice (day 0) was weak and sporadic on the vessels in the lamina propria (200 \times). B, MAdCAM-1 expression of DSS-treated mice on day 7, demonstrating the increased MAdCAM-1 on the vessels in the lamina propria (200 \times). C, MAdCAM-1 expression of DSS-treated mice on day 14, demonstrating the increased MAdCAM-1 on the vessels in the lamina propria as well as in the submucosal layer (200 \times).

ment and averaged in proportion with the length of muscularis mucosa. The number of granulocytes in each segment was counted at high-power field (400 \times) and averaged in proportion with the length of muscularis mucosa.

Immunohistochemistry. Another part of removed colon was fixed for 12 h at 4 $^{\circ}$ C in periodate-lysine-paraformaldehyde. Subse-

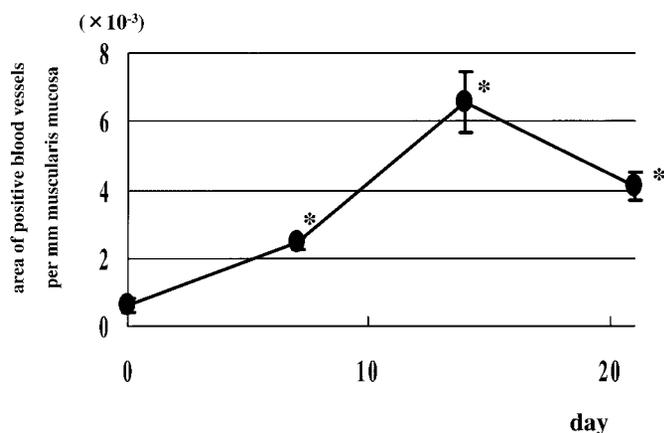


Fig. 3. Changes over time in MAdCAM-1 expression in the lamina propria of distal colon in DSS-treated mice. MAdCAM-1-positive vessels in the lamina propria were determined as area of positively stained vessels per millimeter of muscularis mucosa on days 0, 7, 14, and 21. Results are expressed as the mean \pm S.E. of six animals. * $P < .05$ compared with day 0 (controls).

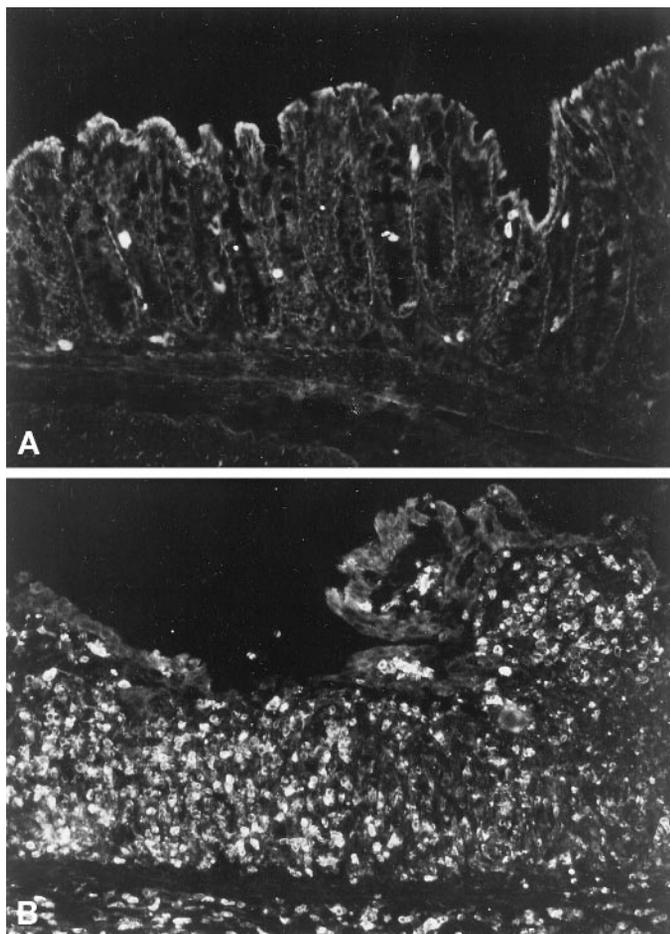


Fig. 4. Immunohistochemical staining of $\beta 7$ -integrin-positive cells in the colonic mucosa of DSS-treated mice. A, $\beta 7$ -integrin-positive cells of DSS-treated mice on day 0, showing only a few cells infiltrating the lamina propria (200 \times). B, $\beta 7$ -integrin-positive cells in DSS-treated mice on day 14, showing a significant cell infiltration in the lamina propria (200 \times).

quently, these specimens were washed and dehydrated for 12 h with PBS containing 10, 15, and 20% sucrose. After fixation, they were embedded in Tissue-Tek O.C.T. compound (Sakura Finetek Inc., Tokyo, Japan) and frozen in liquid nitrogen. Cryostat sections of frozen tissue were cut at 7 μ m. Immunohistochemistry was per-

formed by the labeled streptavidin biotin technique. Primary antibodies used in the immunostaining were monoclonal antibodies (mAbs) that react to MAdCAM-1 (MECA367, rat IgG2a) (Streeter et al., 1988), intercellular adhesion molecule (ICAM)-1 (3-E-2, hamster IgG1), vascular cell adhesion molecule (VCAM)-1 (429, rat IgG2a), $\beta 7$ -integrin (M293, rat IgG2a), CD4 (RM4-5, rat IgG2a), CD8 (53-6.7, rat IgG2a), and CD45R/B220 (RA3-6B2, rat IgG2a) for B-cell marker and MOMA-2 (rat IgG2b) for macrophage marker. These antibodies were obtained from PharMingen (San Diego, CA). The tissue sections were incubated with primary antibodies for overnight at 4 $^{\circ}$ C, and treated with biotinylated goat anti-rat IgG (Southern Biotechnology Associates, Inc., Birmingham, AL) or anti-hamster IgG (KPL, Guilford, UK) for 1 h at room temperature. They were visualized by streptavidin-fluorescein isothiocyanate (FITC) for 30 min. The MAdCAM-1-positive vessels in lamina propria were quantified as area of positively stained vessels per millimeter of muscularis mucosa. The infiltrating cells were expressed as the number of CD4-, CD8-, B220-, and $\beta 7$ -integrin-positive cells per millimeter of muscularis mucosa.

Inhibitory Effect of Anti-MAdCAM-1 mAb on DSS-Induced Colitis. For the study assessing the effect of anti-MAdCAM-1 mAb on DSS-induced colitis, mice were randomized into four groups. Anti-MAdCAM-1 antibody or isotype and species-matched Ig (rat IgG; Chemicon Intern. Inc., Temecula, CA) was administered i.p. at the dose of 200 μ g/mouse. For the daily administration group, DSS-treated mice were administered mAb everyday from day 0 to day 13. As positive control group, DSS-treated mice were administered isotype-matched control IgG everyday from day 0 to day 13. For the early-phase treatment group, DSS-treated mice were administered mAb every day from day 0 to day 6, and for the late-phase treatment group, DSS-treated mice were administered mAb everyday from day 7 to day 13. Histological damage score and immunohistochemical evaluation also were determined in these four groups at day 14. To evaluate the binding of MAdCAM-1 mAb to the colonic mucosa, the localization of infused mAb was examined by immunohistochemistry. Cryostat sections of treated groups were incubated only with a secondary antibody and visualized with streptavidin-FITC.

Statistical Analysis. Results are expressed as mean \pm S.E. The parametric data were statistically analyzed by one-way ANOVA and Fisher's protected least-significant difference test (number of infiltrate cells, area of MAdCAM-1-positive vessels). The nonparametric data were statistically analyzed by Kruskal-Wallis test and Scheffé F test (histological damage score). P values of .05 or less were considered to be statistically significant.

Results

Characterization of DSS-Induced Colitis. Diarrhea was observed after day 14 and bloody stool was observed on day 21 in DSS-treated mice. The earliest histological changes were observed on day 14. The lesions induced by DSS were characterized by slowly progressive colitis with infiltration by a large number of lymphocytes, lymphocyte aggregation in the lamina propria, and crypt distortion on day 14. Loss of entire crypts and erosions were observed on day 21. Histological changes were most common in the distal colon. Histological damage scores were therefore determined for the distal colon. The changes over time in score are shown in Fig. 1. The score was significantly increased after day 14 and further increased on day 21.

As shown in Fig. 2A, constitutive expression of MAdCAM-1 was observed on the small vessels at the bases of crypts in the lamina propria of colonic mucosa in nontreated control mice. However, this expression was weak and sporadic. As shown in Fig. 2B, MAdCAM-1 expression was significantly increased in lamina propria on day 7. MAdCAM-1 expression was further increased on day 14, and at that time it also was

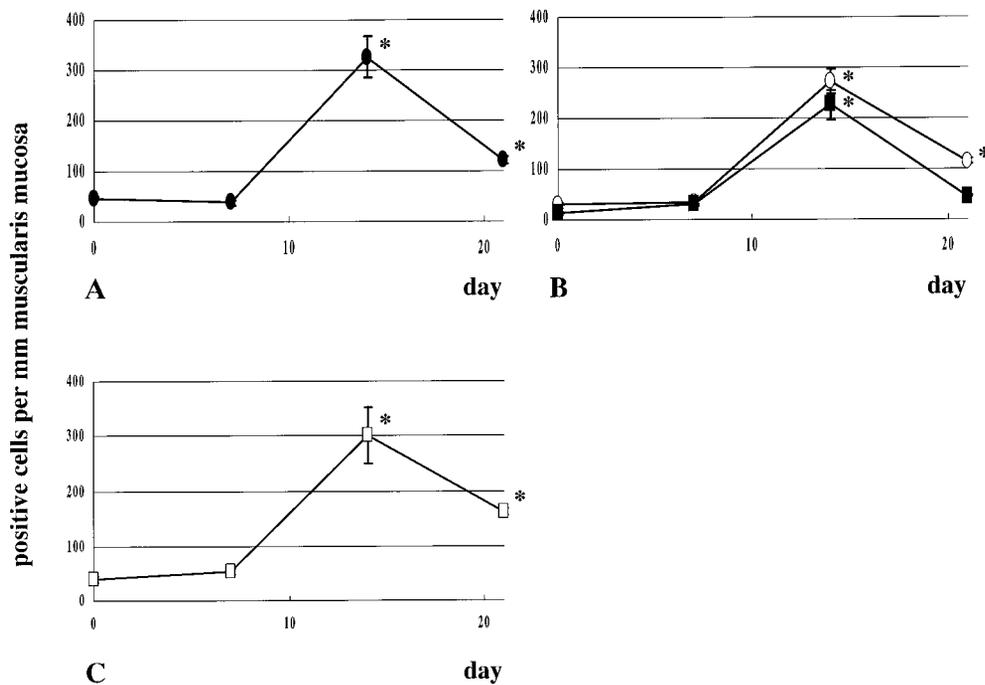


Fig. 5. Changes over time in $\beta 7$ -integrin-, CD4-, CD8-, and B220-positive cells in the lamina propria of distal colon in DSS-treated mice. The number of these cells was determined and expressed as cells per millimeter of muscularis mucosa on days 0, 7, 14, and 21. A, $\beta 7$ -integrin-positive cells (●). B, CD4 (○)- and CD8 (■)-positive cells. C, B220-positive cells (□). Results are expressed as the mean \pm S.E. of six animals. * $P < .05$ compared with day 0 (controls).

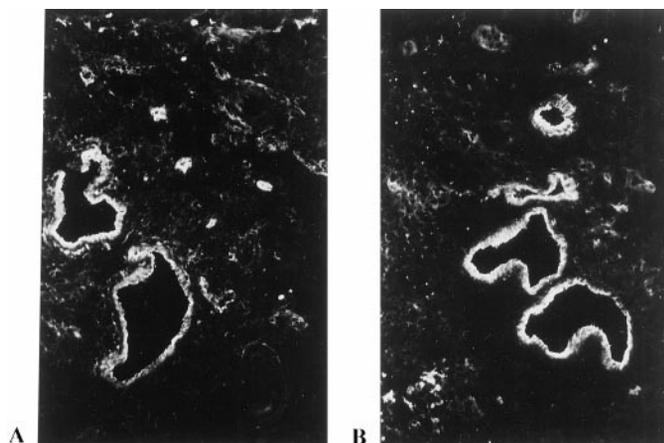


Fig. 6. Immunohistochemical study of ICAM-1 and VCAM-1 expression in the colonic mucosa of DSS-treated mice. A, strong expression of ICAM-1 demonstrated in vascular endothelium of the submucosa on day 21 (400 \times). B, strong expression of VCAM-1 demonstrated in vascular endothelium of the submucosa on day 21 (400 \times).

recognized in vascular endothelium of the submucosal layer in addition to the lamina propria, as seen in Fig. 2C. Figure 3 illustrates the changes over time in area of MAdCAM-1-positive vessels in the lamina propria. It should be noted that MAdCAM-1 expression in the lamina propria vessels was already increased significantly on day 7.

Figure 4 shows the immunohistochemical staining of $\beta 7$ -integrin-positive cells in inflamed colonic mucosa on day 14, and demonstrates many positive cells infiltrating the lamina propria. Figure 5 demonstrates the changes over time in number of $\beta 7$ -integrin-positive cells and the subpopulations of infiltrating lymphocytes in the lamina propria of DSS-treated colon. The number of $\beta 7$ -integrin-positive cells was significantly increased after day 14, although it was not changed on day 7. The number of $\beta 7$ -positive cells was then decreased on day 21, but remained at a significantly greater level than in controls. When we examined the changes in

number of CD4-, CD8-, and B220-positive cells, all of these lymphocyte subpopulations exhibited a profile similar to that of $\beta 7$ -positive cells, with significant increase after day 14, followed by decrease on day 21. The number of CD4-positive cells was greater than that of CD8-positive cells during the course of observation, but there was no significant difference between them. Accumulation of polymorphonuclear leukocytes (PMNLs) also was observed in the inflamed mucosa from day 14, but the number of these cells was significantly smaller than that of lymphocytes (CD4-positive cells versus polymorphonuclear leukocytes on day 21, $115.4 \pm 6.1/\text{mm}$ versus $8.6 \pm 1.1/\text{mm}$; $P < .01$). The number of MOMA-2-positive macrophages was undetectable on day 0, increased at day 7, and significantly increased after day 14 in inflamed mucosa (day 7, $1.8 \pm 0.3/\text{mm}$; day 14, $6.3 \pm 0.9/\text{mm}$; $P < .05$ versus day 0).

No significant expression of ICAM-1 or VCAM-1 was detected in the colonic mucosa of nontreated control mice or DSS-treated mice until day 14. However, strong expression of ICAM-1 and VCAM-1 was demonstrated in vascular endothelium of the colonic submucosa in DSS-treated mice on day 21, as shown in Fig. 6.

Effect of Anti-MAdCAM-1 mAb on DSS-Induced Colitis. The attenuating effect of anti-MAdCAM-1 mAb on chronic DSS-induced colitis was investigated. Because the expression of MAdCAM-1 was significantly increased on day 7, we decided to consider early-phase and late-phase treatments as those before and after day 7, respectively. Figure 7, A and B, compares H&E-stained sections of colonic tissues of control IgG-treated and anti-MAdCAM-1-mAb-treated groups on day 14. As shown in this figure, the degree of inflammation and the extent of mucosal damage were significantly attenuated by daily administration of mAb compared with the positive control group (DSS + control IgG). To confirm the binding of administered MAdCAM-1 mAb to the colonic mucosa, the localization of infused mAb was determined by immunohistochemistry by using only a secondary

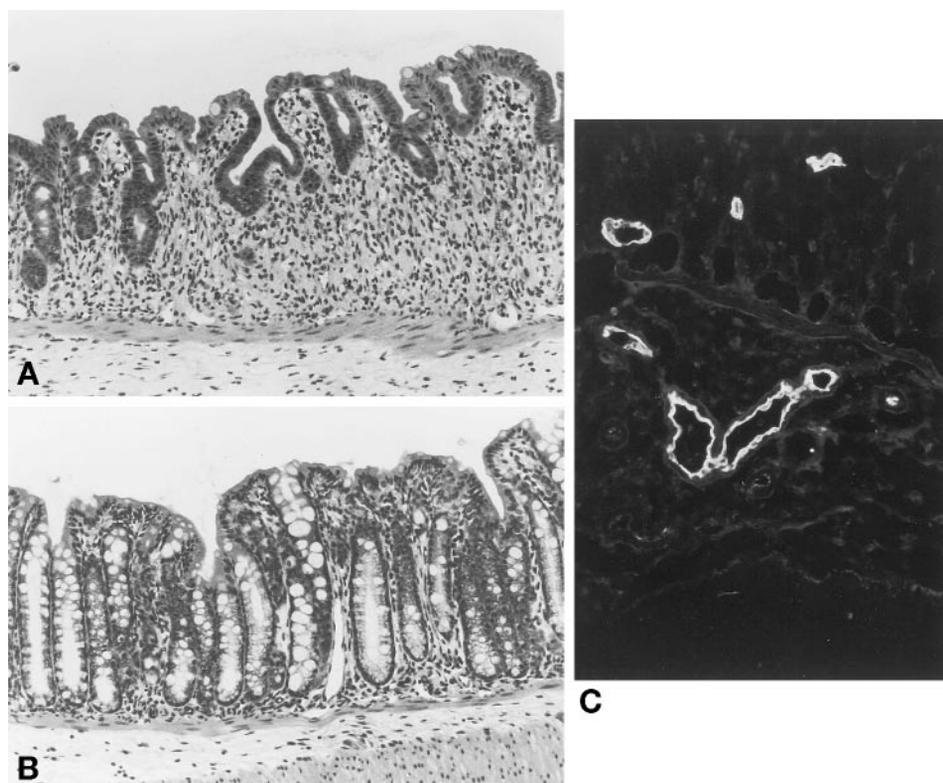


Fig. 7. A, H&E-staining of distal colon 14 days after the treatment with IgG, demonstrating loss of the two-thirds of crypts with the surface epithelium remaining intact (grade 2) (200 \times). B, H&E staining of distal colon of DSS-treated mice 14 days after the continuous treatment with anti-MAdCAM-1 mAb (200 μ g/day), demonstrating the almost intact crypt (grade 0) (200 \times). C, immunohistochemical localization of infused anti-MAdCAM-1 antibody in the colonic mucosa 14 days after the continuous administration to DSS-treated mice. Incubation with a secondary antibody demonstrates clearly that the antibody firmly binds to the endothelium of vessels in the lamina propria and in the submucosa (200 \times).

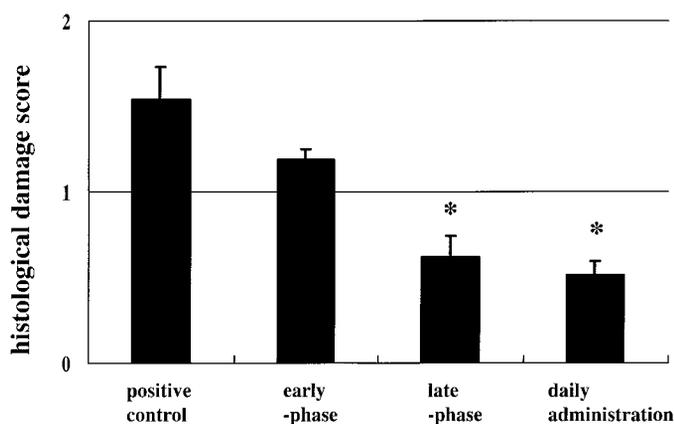


Fig. 8. Effect of administration of anti-MAdCAM-1 antibody on histological damage score of distal colon of DSS-treated mice on day 14. Mice were treated with anti-MAdCAM-1 antibody (200 μ g/day) or isotype-matched control antibody. Positive control: treatment with control antibody for 14 days in DSS-treated mice. Early phase: early-phase treatment group, treated with anti-MAdCAM-1 antibody from day 0 to 6. Late phase: late-phase treatment group, treated with anti-MAdCAM-1 antibody from day 7 to 13. Daily administration: daily administration group, treated with anti-MAdCAM-1 antibody from day 0 to 13. Results are expressed as the mean \pm S.E. of six animals. * P < .05 compared with the positive control.

antibody with streptavidin-FITC. As Fig. 7C demonstrates, MAdCAM-1 mAb was shown to bind to the endothelium of vessels in lamina propria and submucosa. Figure 8 compares the histological damage scores of mAb treatment groups and the positive control group on day 14. The early-phase treatment group exhibited no significant difference from the positive control group in histological damage score, although late-phase treatment and daily administration group significantly prevented histological damage. There was no significant difference between the late-phase group and the daily

administration group in degree of decrease in histological damage score.

We also examined the effect of mAb treatment on number of cells infiltrating the lamina propria in DSS-induced colitis. Figure 9 displays the number of β 7-integrin-positive cells and the numbers of CD4-, CD8-, and B220-positive cells in the lamina propria of DSS-induced colitis on day 14 and the attenuating effects of anti-MAdCAM-1 mAb on number of infiltrating cells with different treatment protocols. Infiltration of β 7-integrin-positive cells was significantly inhibited in either early-phase treatment, late-phase treatment, or daily administration group compared with the positive control group, although both the late-phase treatment and daily administration groups exhibited more significant inhibition of β 7-integrin-positive cell infiltration than did the early-phase treatment group. Anti-MAdCAM-1 mAb also significantly prevented the increased CD4-, CD8-, and B220-positive cell infiltration induced by DSS in either early-phase treatment, late-phase treatment, or daily administration protocol. Similarly, there was no significant difference between the late-phase group and the daily administration group in degree of attenuation of lymphocyte infiltration in the lamina propria.

Discussion

In this study we induced a slowly progressive colitis in BALB/c mice with 5% DSS (mol. wt. 5000). The severity of colitis was highest on the left side of colon, as Cooper et al. (1993) demonstrated; however, in our model, shortening of crypts and increase in number of infiltrating lymphocytes were recognized only on day 14. An important finding of our study is that expression of MAdCAM-1 was already significantly increased in the vessels of lamina propria on day 7,

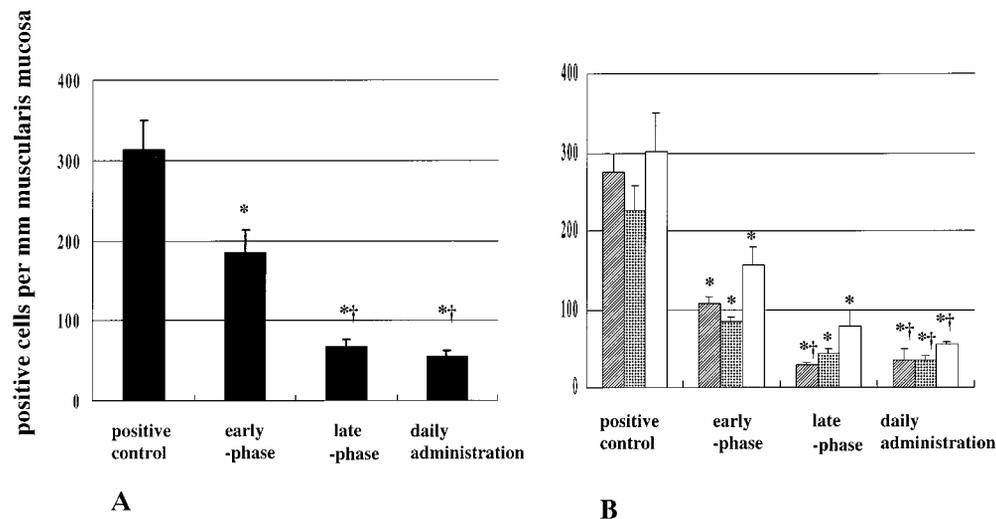


Fig. 9. Effect of administration of anti-MAdCAM-1 antibody on number of (A) $\beta 7$ -integrin-positive cells (■) and (B) CD4 (▨)-, CD8 (▩)-, and B220 (□)-positive cells in the lamina propria of distal colon in DSS-treated mice on day 14. Mice were treated with anti-MAdCAM-1 antibody (200 $\mu\text{g}/\text{day}$) or isotype-matched control antibody. Positive control: treatment with control antibody for 14 days in DSS-treated mice. Early phase: early-phase treatment group, treated with anti-MAdCAM-1 antibody from day 0 to 6. Late phase: late-phase treatment group, treated with anti-MAdCAM-1 antibody from day 7 to 13. Daily administration: daily administration group, treated with anti-MAdCAM-1 antibody from day 0 to 13. Results are expressed as the mean \pm S.E. of six animals. * $P < .05$ compared with the positive control. † $P < .05$ compared with the early-phase treatment.

when histological change was not yet observed. The accumulation of $\beta 7$ -integrin-positive T cells as well as B cells was observed in sites of inflammation after day 14 following significant increase in MAdCAM-1 expression, suggesting that MAdCAM-1 is a major inducer of lymphocyte migration into inflamed colonic mucosa.

Increased expression of MAdCAM-1 has been recognized in other animal models, such as interleukin (IL)-10-deficient mice (Connor et al., 1999), IL-2 knockout mice (McDonald et al., 1997), SCID mice with CD45^{RB}^{high} CD4⁺ T cells (Picarella et al., 1997) and trinitrobenzene sulfonic acid colitis (Viney et al., 1996). In these models, expression of MAdCAM-1 was increased on the vessels in the lamina propria and the submucosal layer. In this study, we also demonstrated that in DSS-induced colitis there was strong expression of MAdCAM-1 in inflamed colon. Although expression of MAdCAM-1 was increased at sites of inflammation in inflammatory bowel diseases, human MAdCAM-1 was recognized in endothelium in gut and lymphoid tissues (Briskin et al., 1997; Souza et al., 1999). In this study, expression of MAdCAM-1 was slight on the small vessels at the bases of crypts in nontreated mice. As for other animal models of experimental colitis, we speculate that expression of MAdCAM-1 was up-regulated on the endothelium of mucosal and submucosal vessels where MAdCAM-1 had already been present.

MAdCAM-1 is induced on a murine endothelial cell line, bEND.3, by tumor necrosis factor (TNF)- α and IL-1-mediated nuclear factor- κB protein in vitro (Sikorski et al., 1993; Takeuchi and Baichwal, 1995). It was previously reported that expression of MAdCAM-1 was time-dependently increased in the colon of wild-type C57BL/6 mice by systemic administration of TNF- α in vivo (Connor et al., 1999). TNF- α and IL-1 are thought to be produced chiefly by activated macrophages (Sartor, 1994), and it was reported that DSS was observed in macrophages in mesenteric lymph nodes and colonic mucosa (Kitajima et al., 1999). Actually, it has been reported that i.p.-administered anti-TNF antibody and anti-IL-1 β -antibody ameliorated DSS-induced colitis (Kojouharoff

et al., 1997; Arai Y et al., 1998). Based on these observations, we speculate that the time-dependent increase in MAdCAM-1 expression in DSS-induced colitis is induced by enhanced TNF- α and IL-1 production from activated macrophages in inflamed mucosa. IL-1 also can stimulate production of IL-2 and interferon- γ by T cells. Subsequent interaction between macrophages and lymphocytes augments inflammation of colonic mucosa with crossregulation of Th1/Th2 responses (Mosmann and Moore, 1991).

We examined the prophylactic effect of anti-MAdCAM-1 antibody treatment on DSS-induced colitis. We found that daily administration of mAb significantly inhibited the progression of colitis. However, we also found that early-phase treatment with mAb did not significantly attenuate histological damage, although it did decrease the number of infiltrating lymphocytes. These results suggest the possibility that MAdCAM-1 plays a key role in the development of DSS-induced colitis by inhibiting lymphocyte migration, but not in the initiation phase of DSS-colitis before up-regulation of MAdCAM-1.

Prophylactic effects of administration of antiadhesion molecules were previously reported for other experimental animal models. Immunoneutralization of MAdCAM-1 and anti- $\beta 7$ -integrin antibody reduced the severity of SCID mice treated with CD45^{RB}^{high} CD4⁺ T cells (Picarella et al., 1997). ICAM-1 and VCAM-1 are other adhesion molecules induced in sites inflammation in colon. Anti-ICAM-1 mAb or anti-ICAM-1 antisense oligonucleotide has been used to prevent acute DSS-induced colitis in rats and mice (Bennett et al., 1997; Taniguchi et al., 1998). Anti-VCAM-1 antibody also was reported to abrogate increased leukocyte adhesion in colonic venules in trinitrobenzene sulfonic acid colitis in rats (Sans et al., 1999). More recently, inhibition of DSS-induced colitis in mice by intracolonic administered antibodies against endothelial leukocyte adhesion molecule-1 or ICAM-1 was reported (Hamamoto et al., 1999). However, in our model expression of neither ICAM-1 nor VCAM-1 was recognized in colonic mucosa of normal mice by immunohis-

tochemistry. Moreover, in the present study, significant expression of ICAM-1 and VCAM-1 was only demonstrated at day 21 of DSS colitis in the vessels of submucosal layer where significant infiltration of PMNLs was seen and significant enhancement of MAdCAM-1 had already occurred. These results suggest that MAdCAM-1 plays a more important role in the induction of DSS-induced colitis than ICAM-1 and VCAM-1, which may play roles mainly in the neutrophil-mediated tissue injury in the later phase of this type of colitis. In clinical studies, levels of ICAM-1, VCAM-1, and MAdCAM-1 were increased in the intestinal mucosa in active ulcerative colitis and Crohn's disease (Nakamura et al., 1993; Jones et al., 1995). Some clinical trials with antiadhesion molecules were previously performed. For example, ICAM-1 antisense oligonucleotide therapy was used to prevent Crohn's disease (Yacyshyn et al., 1998). Because ICAM-1 is generally expressed on the outside of gastrointestinal tract as well, and because MAdCAM-1 is an adhesion molecule more unique to the gut mucosa than ICAM-1, attenuation of MAdCAM-1 may be more specific therapy for colitis. The findings of our present study suggest that anti-MAdCAM-1 therapy may be an effective and organ-specific strategy for treating human inflammatory bowel diseases.

References

- Arai Y, Takanashi H, Kitagawa H and Okayasu I (1998) Involvement of interleukin-1 in the development of ulcerative colitis induced by dextran sulfate sodium in mice. *Cytokine* **10**:890–896.
- Bargatzte RF, Jutila MA and Butcher EC (1995) Distinct roles of L-selectin and $\alpha 4\beta 7$ integrins and LFA-1 in lymphocyte homing to Peyer's patch-HEV in situ: The multistep model confirmed and refined. *Immunity* **3**:99–108.
- Bennett CF, Kornbrust D, Henry S, Stecker K, Howard R, Cooper S, Dutton S, Hall W and Jacoby HI (1997) An ICAM-1 antisense oligonucleotide prevents and reverses dextran sulfate sodium-induced colitis in mice. *J Pharmacol Exp Ther* **280**:988–1000.
- Berlin C, Berg EL, Briskin MJ, Andrew DP, Kilshaw PJ, Holzmann B, Weissman IL, Hamann A and Butcher EC (1993) $\alpha 4\beta 7$ integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. *Cell* **74**:185–195.
- Briskin M, Winsor-Hines D, Shyjan A, Cochran N, Bloom S, Wilson J, McEvoy LM, Butcher EC, Kassam N, Mackay CR, Newman W and Ringler DJ (1997) Human mucosal addressin cell adhesion molecule-1 is preferentially expressed in intestinal tract and associated lymphoid tissue. *Am J Pathol* **151**:97–110.
- Butcher EC (1991) Leukocyte-endothelial cell recognition: Three (or more) steps to specificity and diversity. *Cell* **67**:1033–1036.
- Connor EM, Eppihimer MJ, Morise Z, Granger DN and Grisham MB (1999) Expression of mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in acute and chronic inflammation. *J Leukoc Biol* **65**:349–355.
- Cooper HS, Murthy SN, Shah RS and Sedergran DJ (1993) Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest* **69**:238–249.
- Elson CO, Sartor RB, Tennyson GS and Riddell RH (1995) Experimental models of inflammatory bowel disease. *Gastroenterology* **109**:1344–1367.
- Faveeuw C, Gagnerault MC and Lepault F (1994) Expression of homing and adhesion molecules in infiltrated islets of Langerhans and salivary glands of nonobese diabetic mice. *J Immunol* **152**:5969–5978.
- Hamamoto N, Maemura K, Hirata I, Murano M, Sasaki S and Katsu K (1999) Inhibition of dextran sulphate sodium (DSS)-induced colitis in mice by intracolonic administered antibodies against adhesion molecules (endothelial leukocyte adhesion molecule (ELAM-1) or intercellular adhesion molecule-1 (ICAM-1)). *Clin Exp Immunol* **117**:462–468.
- Hamann A, Andrew DP, Jablonski-Westrich D, Holzmann B and Butcher EC (1994) Role of $\alpha 4$ -integrins in lymphocyte homing to mucosal tissue in vivo. *J Immunol* **152**:3282–3293.
- Hanninen A, Taylor C, Streeter PR, Stark LS, Sarte JM, Shizuru JA, Simell O and Michie SA (1993) Vascular addressins are induced on islet vessels during insulinitis in nonobese diabetic mice and are involved in lymphoid cell binding to islet endothelium. *J Clin Invest* **92**:2509–2515.
- Jones SC, Banks RE, Haidar A, Gearing AJ, Hemingway IK, Ibbotson SH, Dixon MF and Axon AT (1995) Adhesion molecules in inflammatory bowel disease. *Gut* **36**:724–730.
- Kitajima S, Takuma S and Morimoto M (1999) Tissue distribution of dextran sulfate sodium (DSS) in the acute phase of murine. *J Vet Med Sci* **61**:67–70.
- Kojouharoff G, Hans W, Obermeier F, Mannel DN, Andus T, Scholmerich J, Gross V and Falk W (1997) Neutralization of tumor necrosis factor (TNF) but not of IL-1 reduces inflammation in chronic dextran sulfate sodium-induced colitis in mice. *Clin Exp Immunol* **107**:353–358.
- McDonald SA, Palmen MJ, Van Rees EP and MacDonald TT (1997) Characterization of the mucosal cell-mediated immune response in IL-2 knockout mice before and after the onset of colitis. *Immunology* **91**:73–80.
- Miura S, Tsuzuki Y, Kurose I, Suematsu M, Shigematsu T, Kimura H, Higuchi H, Serizawa H, Yagita H, Okumura K, Granger DN and Ishii H (1996) Endotoxin stimulates lymphocyte-endothelial interactions in rat intestinal Peyer's patches and villus mucosa. *Am J Physiol* **271**:G282–G292.
- Mosmann TR and Moore KW (1991) The role of IL-10 in crossregulation of TH1 and TH2 responses. *Immunol Today* **12**:49–53.
- Nakamura S, Ohtani H, Watanabe Y, Fukushima K, Matsumoto T, Kitano A, Kobayashi K and Nagura H (1993) In situ expression of the cell adhesion molecule in inflammatory bowel disease. Evidence of immunologic activation of vascular endothelial cells. *Lab Invest* **69**:77–85.
- Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y and Nakaya R (1990) A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* **98**:694–702.
- O'Neill JK, Butter C, Baker D, Gschmeissner SE, Kraal G, Butcher EC and Turk JL (1991) Expression of vascular addressins and ICAM-1 by endothelial cells in the spinal cord during chronic relapsing experimental allergic encephalomyelitis in the Biozzi AB/H mouse. *Immunology* **72**:520–525.
- Picarella D, Hurlburt P, Rottman J, Shi X, Butcher E and Ringler DJ (1997) Monoclonal antibodies specific for $\beta 7$ integrin and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) reduce inflammation in the colon of scid mice reconstituted with CD45RB^{high} CD4+ T cells. *J Immunol* **158**:2099–2106.
- Sans M, Panés J, Ardite E, Elizalde JI, Arce Y, Elena M, Palacín A, Fernández-Checa JC, Anderson DC, Lobb R and Piqué JM (1999) VCAM-1 and ICAM-1 mediate leukocyte-endothelial cell adhesion in rat experimental colitis. *Gastroenterology* **116**:874–883.
- Sartor RB (1994) Cytokines in intestinal inflammation: Pathophysiological and clinical considerations. *Gastroenterology* **106**:533–539.
- Shimizu Y, Newman W, Tanaka Y and Shaw S (1992) Lymphocyte interactions with endothelial cells. *Immunol Today* **13**:106–112.
- Sikorski EE, Hallmann R, Berg EL and Butcher EC (1993) The Peyer's patch high endothelial receptor for lymphocytes, the mucosal vascular addressin, is induced on a murine endothelial cell line by tumor necrosis factor- α and IL-1. *J Immunol* **151**:5239–5250.
- Souza HS, Elia CC, Spencer J and MacDonald TT (1999) Expression of lymphocyte-endothelial receptor-ligand pairs, $\alpha 4\beta 7$ /MAdCAM-1 and OX40/OX40 ligand in the colon and jejunum of patients with inflammatory bowel disease. *Gut* **45**:856–863.
- Springer T (1994) Traffic signals for lymphocyte recirculation and leukocyte emigration: The multistep paradigm. *Cell* **76**:301–314.
- Streeter PR, Berg EL, Rouse BT, Bargatzte RF and Butcher EC (1988) A tissue-specific endothelial cell molecule involved in lymphocyte homing. *Nature (Lond)* **331**:41–46.
- Takeuchi M and Baichwal VR (1995) Induction of the gene encoding mucosal vascular addressin cell adhesion molecule-1 by tumor necrosis factor- α is mediated by NF- κ B proteins. *Proc Natl Acad Sci USA* **92**:3561–3565.
- Taniguchi T, Tsukada H, Nakamura H, Kodama M, Fukuda K, Saito T, Miyasaka M and Seino Y (1998) Effects of the anti-ICAM-1 monoclonal antibody on dextran sulfate-induced colitis in rats. *J Gastroenterol Hepatol* **13**:945–949.
- Viney JL, Jones S, Chiu HH, Lagrimas B, Renz ME, Presta LG, Jackson D, Hillan KJ, Lew S and Fong S (1996) Mucosal addressin cell adhesion molecule-1; A structural and functional analysis demarcates the integrin binding motif. *J Immunol* **157**:2488–2497.
- Yacyshyn BR, Bowen-Yacyshyn MB, Jewell L, Tami JA, Bennett CF, Kisner DL and Shanahan WR Jr (1998) A placebo-controlled trials of ICAM-1 antisense oligonucleotide in the treatment of Crohn's disease. *Gastroenterology* **114**:1133–1142.

Send reprint requests to: Soichiro Miura, M.D., Professor, Second Department of Internal Medicine, National Defense Medical College, 3-2 Namiki, Tokorozawa City, Saitama 359-8513, Japan.