Amelioration of Murine Experimental Colitis by Inhibition of Mucosal Addressin Cell Adhesion Molecule-1

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ABSTRACT

Mucosal addressin cell adhesion molecule-1 (MAdCAM-1) is a cell 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 $\beta$-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 $\beta$-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 adressin 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 lymphocytes 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-

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

Immunohistochemistry. Another part of removed colon was fixed for 12 h at 4°C in periodate-lysine-paraformaldehyde. Subse-
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), β7-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°C, and treated with biotinylated goat anti-rat IgG (Southern Biotechnology Associates, Inc., Birmingham, AL) or anti-hamster IgG (KPL, Gaithersburg, MD) 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 β7-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 μg/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 ± 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 Scheffe 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...
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 laminal papillary vessels was already increased significantly on day 7.

Figure 4 shows the immunohistochemical staining of β7-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 β7-integrin-positive cells and the subpopulations of infiltrating lymphocytes in the lamina propria of DSS-treated colon. The number of β7-integrin-positive cells was significantly increased after day 14, although it was not changed on day 7. The number of β7-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 β7-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 ± 6.1/mm versus 8.6 ± 1.1/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 ± 0.3/mm; day 14, 6.3 ± 0.9/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
antibody with streptavidin-FITC. As Fig. 7C demonstrates, MAdCAM-1 mAb was shown to bind to the endothelium of vessels with the surface epithelium remaining intact (grade 0) (200×). 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×).

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 β7-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 β7-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 β7-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,
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 (Connor et al., 1999), 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-1β-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-β7-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-VCAM-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 intracoelomically 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-
References


