Cytokine and endothelial cell adhesion molecule expression in interleukin-10-deficient mice

SHIGEYUKI KAWACHI,1 STEPHEN J ENNINGS,2 JULIAN PANES,3 ADAM COCKRELL,3 F. STEPHEN LAROUX,3 LAURA GRAY,1 MICHAEL PERRY,4 HENRY VAN DER HEYDE,2 EDWARD BALISH,5 D. NEIL GRANGER,1 ROBERT A. SPECIAN,1 AND MATTHEW B. GRISHAM1

Departments of 1Molecular and Cellular Physiology and 2Microbiology and Immunology, Louisiana State University Medical Center, Shreveport, Louisiana 71130; 2Department of Gastroenterology, University of Barcelona, Barcelona, Spain; 4School of Physiology and Pharmacology, University of New South Wales, Sydney, Australia; and 5Department of Surgery, University of Wisconsin, Madison, Wisconsin 53706

INFLAMMATORY BOWEL DISEASE (IBD) is a recurrent inflammation of the small and/or large bowel of unknown etiology. Increasingly, experimental and clinical data are consistent with the hypothesis that deletion of IL-10 results in the sustained production of proinflammatory cytokines, leading to the upregulation of adhesion molecules and infiltration of mononuclear and polymorphonuclear leukocytes into the cecal and colonic intestitium.

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systematic or quantitative information is available regarding the relationship between cytokine and ECAM expression in the IL-10−/− model of colitis. Thus the objectives of this study were to quantify message levels for a variety of different Th1 and Th2 cytokines and ECAM message and protein expression in the colons of healthy wild-type and IL-10−/− mice with distal bowel disease.

**MATERIALS AND METHODS**

**Animals**

IL-10−/− mice were generated by gene-targeting deletion in embryonic stem cells as described by Kuhn et al. (31). The mice used in this study were bred onto a 129SvEv back- ground. When raised in specific pathogen-free conditions, IL-10−/− mice develop spontaneous colitis at 8–12 wk of age. Wild-type mice (129SvEv) were used as controls at 8–12 wk of age and were obtained from Taconic Labs (Germantown, NY). Development of colitis was assessed as previously described by Berg et al. (4), using serum amyloid A protein levels as an indicator of ongoing inflammation.

**Colonic RNA Isolation and Quantification of mRNA by RNase Protection Assay**

Colons were removed, cleaned of intestinal contents, and weighed, and total length was determined. The tissue was then divided into proximal and distal colon. Total RNA was isolated from the tissues using TRIzol reagent (GIBCO BRL), according to the manufacturer’s instructions. RNase protection assay was performed using [32P]UTP-labeled MCK2b and MCK3b RiboQuant probe sets (Pharmingen) for the different Th1 and Th2 cytokines. ICAM-1, vascular cell adhesion molecule-1 (VCAM-1), and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) message levels were quantified using a custom probe set obtained from Pharmingen (San Diego, CA). Probe synthesis, hybridization, RNase treatment, gel preparation, and electrophoresis were all performed according to the manufacturer’s instructions. Autoradiographs of the resulting protected species were processed into digital images analyzed and quantified using ImageQuant software (Molecular Dynamics). The relative levels of mRNA for the two different cytokines and ECAMs were determined from the ratio of the density obtained for each cytokine to the density obtained for the mRNA of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase. Multiple exposures of each reaction were generated to ensure accuracy of the comparison of the two signals under different signal density conditions.

**ECAM Expression In Vivo**

Monoclonal antibodies used for experiments. The dual radiolabeled monoclonal antibody (MAb) technique was used to quantify ICAM-1, ICAM-2, VCAM-1, and MAdCAM-1 expression in different vascular beds (12, 19, 27), YN-1, a rat IgG2b directed against mouse ICAM-1 (Bayer, West Haven, CT); 3C4 (MIC2/4), a rat IgG2a directed against mouse ICAM-2 (Pharmingen); MK1.9.1, a rat IgG1 targeted against mouse VCAM-1 (Bayer), and MECA-367, a rat IgG2a targeted against mouse MAdCAM-1 (Pharmingen) were used as the MAbs. P-23, a murine IgG1 against human P-selectin (Pharmacia-Upjohn, Kalamazoo, MI), was used as the control nonbinding MAb.

The binding MAbs directed against ICAM-1, ICAM-2, VCAM-1, and MAdCAM-1 were radiolabeled with 125I (DuPont NEN, Boston, MA), whereas the nonbinding MAb (P-23) was labeled with 131I. Radiolodination of the MAbs were performed by the iodogen method as previously described (12, 19, 27).

**Animal procedures.** At ~12 wk of age, both wild-type and IL-10−/− mice were anesthetized with ketamine hydrochloride (150 mg/kg im) and xylazine (7.5 mg/kg im), and the right jugular vein and carotid artery were cannulated with polyethylene tubing.

For assessing the ICAM-1 expression, a mixture of 10 µg of 125I anti-ICAM-1 MAb and 40 µg of unlabeled anti-ICAM-1 MAb was given with an amount of 131I-labeled P-23 necessary to ensure a total 131I-injected activity of 400,000–600,000 cpm through the jugular vein cannula (total vol 200 µl). In the ICAM-2 experiments, a mixture of 10 µg of 125I-labeled anti-ICAM-2 MAb and 60 µg of unlabeled anti-ICAM-2 MAb was given with an appropriate amount of 131I P-23 (400,000–600,000 cpm). In the VCAM-1 experiments, a mixture of 10 µg of 125I-labeled anti-VCAM-1 MAb and 20 µg of unlabeled anti-VCAM-1 MAb was administered with an appropriate amount of 131I-labeled P-23 through the jugular vein. In the MAdCAM-1 experiments, only 10 µg of 125I-labeled anti-MAdCAM-1 MAb were administered with an appropriate amount of 131I-labeled P-23 through the jugular vein. These dosages were selected on the basis of pilot studies demonstrating optimum activity and receptor saturation in the tissues examined under constitutive and stimulated conditions.

A blood sample was taken 5 min after MAb injection. Bicarbonate-buffered saline was isovolumically exchanged by infusion of buffer through the jugular vein and simultaneous withdrawal of blood and buffer from the artery. After the vascular system was completely flushed with bicarbonate-buffered saline, the small intestine, cecum, large intestine, and a variety of other tissues were harvested and weighed.

Calculation of ECAM expression. A 14800 Wizard 3 gamma counter (Wallac, Turku, Finland), with automatic correction for background activity and spillover, was used to count 125I and 131I activities in each organ and in a 100-µl plasma sample. The injected activity in each experiment was calculated by counting a 4-µl sample of mixture containing the radiolabeled MAbs. The amount of radioactivity remaining in the tube used to mix the MAbs and the syringe used to inject the mixture was subtracted from the total calculated injected activity. The accumulated activity of each MAb in an organ was expressed as the percentage of the injected dose (%ID) per gram of tissue. ECAM expression was calculated by subtracting the nonspecific tissue accumulation of MAb from the accumulation of binding MAbs as follows: VCAM-1 expression = (%ID/g for 125I) – (%ID/g for 131I) × (%ID for 131I in plasma)/(%ID for 131I in plasma). This formula corrects the tissue accumulation of nonbinding MAb for the relative plasma levels of both binding and nonbinding MAbs to estimate the nonspecific tissue accumulation of MAb. This value, expressed as %ID/g, was converted to µg MAb/g tissue by multiplying the above value by the total injected binding MAb (in µg), divided by 100.

**Immunohistochemical Assessment of ECAM Expression**

Separate groups of animals (n = 3 for each group) were anesthetized with ketamine hydrochloride (150 mg/kg im) and xylazine (7.5 mg/kg im), and the right jugular vein and carotid artery were cannulated with polyethylene tubing. For assessing the immunolocalization of the four ECAMs, the same total amount of MAb against the different ECAMs was used as in the dual radiolabeled MAb technique described above. However, the MAbs were not radiolabeled; 50 µg of ICAM-1 MAb, 70 µg of ICAM-2 MAb, 30 µg of VCAM-1 MAb,
and 10 µg of MAdCAM-1 MAb were infused in each experiment. Five minutes after MAb injection, the vasculature was completely flushed with bicarbonate-buffered saline, and tissues were excised exactly as described for the radiolabeled MAb experiments.

The colons were divided into proximal and distal portions, and the contents were removed by gentle flushing with Zamboni’s fixative using a syringe and a 30-gauge needle. The samples were then placed in Zamboni’s fixative at 4°C overnight. The following day, the samples were placed in 80% ETOH to remove picric acid from the solution, cleared in DMSO, and washed three times in PBS. The samples were then stored in PBS containing 30% sucrose and 0.01% sodium azide at 4°C overnight. Samples were placed in OCT embedding medium, and the blocks were frozen with isopentane chilled in liquid nitrogen. Frozen samples were sectioned in a cryostat at 10-µm thickness and collected on poly-L-lysine-coated slides. The slides were then dried over P2O5 in a vacuum desiccator for 30 min.

Colon sections were blocked with 10% normal donkey serum and then incubated with a donkey anti-rat secondary antibody against ECAMs conjugated to Cy3 fluorochrome in a humid box for 1 h. The slides were washed in PBS three times for 10 min each time, and the sections around and on the back were dried before mounting. Slides were then mounted in Mowiol-glycerol containing 2.5% 1,4-diazabicyclo[2.2.2]octane (pH 8.5). Sections were also stained with hematoxylin and eosin for standard observation of histology.

Histopathology

Colon sections from three representative wild-type and IL-10−/− mice were recovered from animals at necropsy and fixed in Zamboni’s fixative. The tissues were rinsed in 0.1 M phosphate buffer and dehydrated with 95% ethanol. The tissues were then embedded in glycol methacrylate (JB-4; Polysciences, Warrington, PA). Sections (1–2 µm) were cut on glass knives, stained with hematoxylin and eosin, dried on a warming plate, and mounted in Permount. Samples were evaluated, and photographs were taken with a SenSys digital camera and processed with Metamorph.

Statistical Analysis

All values are presented as means ± SE. Data were analyzed using standard statistical analyses, i.e., one-way ANOVA and a paired and unpaired Student’s t-test. Statistical significance was set at P < 0.05.

RESULTS

Histopathology

Of the IL-10−/− mice, 100% developed moderate to severe inflammation with marked thickening of the intestinal wall, cecum, and proximal colon at 3 mo of age. Corresponding to this spontaneous colitis was a dramatic increase in serum amyloid A protein levels (73 vs. 2,381 µg/ml for wild-type vs. IL-10−/− mice; P < 0.01). The colon in IL-10−/− mice was significantly longer than that of wild-type mice (9.23 ± 0.18 vs. 7.02 ± 0.22 cm), and the weight of colon in IL-10−/− mice was significantly heavier than that of wild-type mice (0.57 ± 0.03 vs. 0.20 ± 0.04 g). Histopathology revealed moderate to severe inflammation of the colon. The colonic mucosa exhibited epithelial hyperplasia with infiltration of chronic inflammatory cells such as lymphocytes, plasma cells, and macrophages but also contained small-to-moderate numbers of neutrophils. This infiltrate was present in both the mucosa and the submucosa. Transmural inflammation, multiple small superficial erosions of the mucosa covered by inflammatory exudate, mucus, and cellular debris, and crypt abscesses were present as well.

Cytokine mRNA Expression

The pattern of cytokine mRNA expression in wild-type and colitic IL-10−/− mice revealed enhanced expression of IL-1α, IL-1β, interleukin-1 receptor antagonist, and IL-18 as well as IL-6, IFN-γ, TNF-α, and lymphotoxin (LT)-β compared with their healthy controls (Fig. 1). LT-α was present at low levels in colitic colons yet significantly above the levels detected in the healthy wild-type mice (Fig. 1). IL-10 mRNA was totally absent in colitic IL-10−/− mice, as expected; however, TGF-β1 mRNA levels were increased approximately threefold (Fig. 1). Colitic IL-10−/− mice also expressed low but detectable levels of mRNA for the IL-12 p35 and p40 subunits (Fig. 1).

ECAM Expression

Colonic message levels of ICAM-1, VCAM-1, and MAdCAM-1 in colitic IL-10−/− mice were found to be significantly increased by 10–50–300-fold, respectively, compared with their wild-type controls (Fig. 2). Corresponding to these large and significant increases in ECAM mRNA was enhanced surface expression of colonic ICAM-1, VCAM-1, and MAdCAM-1 (Fig. 3). Colonic VCAM-1 and MAdCAM-1 expression were enhanced eight- and fourfold, respectively, whereas ICAM-1 surface expression was increased by 50% (Fig. 3). Immunolocalization of each ECAM using the same method described for the radiolabeled MAb technique revealed enhanced vascular staining of ICAM-1, VCAM-1, and MAdCAM-1 in colitic mice compared with their wild-type controls (Fig. 4). Constitutive expression of VCAM-1 in healthy wild-type mice was below the sensitivity of the immunolocalization method; however, we were able to observe enhanced vascular expression of VCAM-1 in the IL-10−/− mice (Fig. 4). We observed low levels of constitutive expression of ICAM-1 that increased in submucosal and mucosal vessels in the IL-10−/− mice (Fig. 4). Enhanced vascular expression of MAdCAM-1 was also observed in colitic mice; however, the staining pattern for MAdCAM-1 was quite different from those of the other ECAMs. We observed staining of large mucosal and submucosal vessels that appeared approximately two to three times larger than those vessels from wild-type animals with constitutive expression of MAdCAM-1 (Fig. 4). Furthermore, the staining appeared to be functional in nature. Because ICAM-2 is constitutively expressed and not upregulated in response to a variety of proinflammatory mediators and/or cytokines (40), we quantified this ECAM to assess vascular surface area in wild-type vs. colitic mice. We found, using either the dual radiolabel or immunohistochemical techniques, that ICAM-2 ex-
Fig. 1 Relative message levels of different colonic cytokines in wild-type and interleukin-10-deficient (IL-10-/-) mice normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Three representative wild-type mice (filled bars) and three colitic IL-10-/- mice (open bars) were used for these studies. Relative increases in cytokine mRNA were determined from ratio of density obtained for each cytokine to density determined for housekeeping gene GAPDH. Specific protocols are described in MATERIALS AND METHODS. Data are expressed as mean values. LT-α or -β, lymhotoxin-α or -β; TNF-α, tumor necrosis factor-α; IL-1Ra, interleukin-1 receptor antagonist; IFN-γ, interferon-γ; TGF, transforming growth factor; MIF, macrophage migratory inhibitory factor.

Fig. 2 Colonic message levels of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in wild-type and IL-10-/- mice. Specific methods for RNA isolation and RNase protection assay are described in MATERIALS AND METHODS. L32, ribosomal structural protein. B: filled bars, wild-type mice; open bars, IL-10-/- mice.
METHODS. ECAMs were quantified in mice at 3 mo of age. Vascular surface expression of the different ECAMs was quantified using the dual radiolabeled monoclonal antibody (MAb) technique described in MATERIALS AND METHODS. ECAMs were quantified in mice at 3 mo of age. A: ICAM-1. B: VCAM-1. C: MAdCAM-1. D: ICAM-2. *P < 0.05 vs. wild type.

DISCUSSION

The distal bowel is continuously exposed to numerous environmental antigens and bacterial products, resulting in a constant threat of a potentially dangerous and debilitating inflammatory response. For this reason, immune responses of the intestine and colon are under very strict regulatory control (17, 43, 54). A variety of recently developed mouse models have provided important insights into the mechanisms that control the immune response in the intestinal tract and the aberrations that lead to a disease state. Data obtained from a variety of different studies using genetically engineered or immune-manipulated models of colitis suggest a regulatory network in which the cytokines act through proteins that control expression of potentially damaging cytokines, such as IFN-γ and TNF-α (17, 43, 48). One of the best-studied models of spontaneous and chronic colitis is the IL-10−/− model bred to the 129 strain background (4, 47, 48). All of these mice develop colitis when housed under conventional conditions, due principally to the absence of the major regulatory cytokine IL-10 (4, 47, 48). Studies with this model, as well as others, suggest that chronic colitis results from a dysregulated immune response to components of the normal gut flora (43, 48). One of the earliest manifestations of this uncontrolled activation of T cells within the cecal and colonic interstitium is the sustained production of proinflammatory mediators such as the Th1 and macrophage-derived cytokines. In the present study, we found large and significant increases in IL-1α, IL-1β, IL-6, IFN-γ, and TNF-α mRNA in colons of IL-10−/− mice compared with their healthy controls (Fig. 1). Each of these cytokines was strongly associated with ongoing colitis. These findings are consistent with previous reports (2, 7, 14, 38, 39, 44) implicating IFN-γ and TNF-α as major mediators in the pathophysiology of chronic colitis.

However, the patterns of other cytokine species were unexpected. In the IL-10−/− colitis model, it was expected that no IL-10 mRNA would be detected, and this was found to be true. This finding is also consistent with the concept that IL-10 production is essential for the development and expression of function of regulatory CD4+ T cells, designated Tr-1 (25). Interestingly, it has been reported that IL-10 is an important growth and differentiation factor for Tr-1 CD4+ T cells, which exert their regulatory function principally through the production of the inhibitory cytokine TGF-β (25). However, the level of mRNA for TGF-β1 was significantly elevated, yet profound disease was still present. These results suggest that TGF-β alone, in the absence of IL-10, or in the presence of elevated levels of other cytokines, is not sufficient to limit the development of IBD. The cellular origin of TGF-β in this model is not known. It is likely that it is derived from a non-T lymphocyte source within the site of inflammation.

Another unexpected observation made in the present study was the divergence in expression of LT-α and LT-β mRNA species. In healthy wild-type mice, low but discernible levels of both mRNA species are present. However, in colitic mice, LT-β mRNA was very high, whereas LT-α mRNA was very low to undetectable. This was an unexpected observation, since the mature, secreted form of the lymphotoxin molecule is composed of a heterotrimeric complex consisting of one chain of LT-α and two chains of LT-β (1). The absence of the LT-α mRNA species may indicate that, during chronic inflammation, functional LT is not secreted by activated CD4+ T cells within the site of inflammation. However, LT plays a critical role in the initiation of disease, since IBD does not develop in mice in which the signaling via the LT-β receptor (LT-βR) is blocked by a soluble
LT-βR-Ig fusion molecule (34). A potential consequence of the uncontrolled production of Th1 and macrophage-derived cytokines is the transcriptional activation of ECAM expression within the colonic microvasculature. A number of different experimental and clinical studies (24) suggest that leukocyte-endothelial cell interactions play a critical role in initiating and/or perpetuating the chronic gut inflammation observed in experimental as well as human IBD. The microvasculature occupies a critical position in the inflammatory response by virtue of its ability to differentially regulate the infiltration of specific populations of leukocytes. In this respect, the postcapillary venules may define the type of inflammatory cell that may gain access to the

Fig. 4. Immunolocalization of different ECAMs in colons obtained from wild-type (wt) and IL-10−/− mice. At 3 mo of age, unlabeled rat anti-mouse MAb specific for the different ECAMs was injected (iv), and tissues were processed as described in MATERIALS AND METHODS. Frozen sections were stained with donkey anti-rat secondary antibody conjugated to Cy3 fluorochrome. Note vascular immunolocalization of each MAb, indicating little or no staining of extravascular cells and tissue (magnification, ×100).
colonic interstitium and dictate whether an acute and/or chronic inflammation will ensue.

In the present study, we demonstrate that chronic enterocolitis in IL-10<sup>−/−</sup> mice is associated with enhanced expression of ICAM-1, VCAM-1, and MadCAM-1 message and protein in the colon (Figs. 2 and 3). The vascular localization of these ECAMs was confirmed using immunohistochemistry (Fig. 4).

ICAM-1 and ICAM-2 are ECAMs, which are members of the immunoglobulin supergene family (11). ICAM-1 contains five immunoglobulin-like extracellular domains of which the first NH2-terminal immunoglobulin-like domain recognizes CD11a/CD18 and the third immunoglobulin-like domain recognizes CD11b/CD18 (15). ICAM-1 is basally expressed on endothelial cells, and its expression is increased in response to activation of endothelial cells with certain Th1 and/or macrophage-derived cytokines or bacterial products.

Maximal expression of ICAM-1 is achieved within 4–8 h after activating the endothelial cells and is associated with maximal levels of leukocyte adherence. Indeed, we observed a significant increase in both message levels as well as surface expression of ICAM-1 in the IL-10<sup>−/−</sup> colitic mice (Figs. 2 and 3). The disparity between the magnitude of the increase in mRNA vs. surface expression of ICAM-1 (10-fold vs. 50%, respectively) most probably reflects the large increase in infiltrating ICAM-1-containing cells (e.g., lymphocytes, monocytes), whereas the more modest increase in ICAM-1 surface expression reflects increases in ICAM-1 only on vascular endothelium (Fig. 6). Several different reports (29, 35, 37, 41) have demonstrated enhanced staining for ICAM-1 in biopsies obtained from patients with active ulcerative colitis and Crohn’s disease. Furthermore, recent experimental and clinical studies (3, 58) have demonstrated that infusion of an antisense oligonucleo-
tide directed against ICAM-1 message produces clinical improvement in a mouse model of colitis and in steroid-resistant Crohn’s disease patients.

ICAM-2 is a truncated form of ICAM-1 containing only two immunoglobulin-like extracellular domains. Like ICAM-1, ICAM-2 also is basally expressed on endothelial cells, but its expression is not increased in response to cytokine exposure (40). We found that the expression of ICAM-2 was similar between IL-10−/− and wild-type mice, suggesting that the density of the colonic vascular bed (i.e., vascular surface area) was similar between colitic and control mice (Figs. 3 and 4). Together, these data suggest that the observed increases in ICAM-1, VCAM-1, and MAdCAM-1 expression are due to increases in expression on the vascular endothelium rather than increases in colonic vascular density. VCAM-1 is an inducible ECAM, which is known to mediate the firm adhesion of monocytes and lymphocytes to endothelial cells (22). Thus this ECAM may act to promote adhesion and immigration of chronic inflammatory cells into colonic interstitium (22). Proinflammatory cytokines, such as TNF-α and IL-1β, both of which are upregulated in this model, induce VCAM-1 expression in various organs via activation of nuclear factor-κB (24). It is known that lymphocytes, monocytes, and eosinophils possess a β1 integrin, called very late activation antigen-4 (αβ1), which binds to VCAM-1 (and MAdCAM-1) (22). The kinetics of VCAM-1 expression on vascular endothelial cells closely resemble those seen with ICAM-1. Although a great deal of interest has been generated regarding the possibility that VCAM-1 may be important in lymphocyte infiltration in IBD, several investigators (29, 30, 37) have failed to demonstrate enhanced expression of VCAM-1 in biopsy specimens obtained from patients with active colitis. These observations are particularly surprising in view of data obtained in the present study and in three recent reports (5, 6, 26), which demonstrated that primary cultures of microvascular endothelial cells isolated from human intestine and colon respond to different proinflammatory cytokines with enhanced surface expression of VCAM-1. The reasons for these differences between experimental and human IBD are not known but may represent the inherent variability known to be associated with immunohistochemistry compared with the more objective, radiolabeled MAb method to quantify ECAM expression in vivo. Alternatively, the quality of the antibodies used for the immunohistochemical studies may represent an important determinant for accurate determination of VCAM-1. In fact, our data would suggest that the dual radiolabeled MAb technique provides the necessary sensitivity to accurately determine low-level constitutive expression of VCAM-1 in the absence of any immunohistochemical evidence for VCAM-1-positive vessels in healthy controls (Fig. 4). One of the more interesting findings in the present study was the localization of MAdCAM-1 to dilated mucosal and submucosal vessels in the colon, which appeared to be junctional in nature (Fig. 4). MAdCAM-1 was first described as an endothelial cell surface molecule that is selectively expressed in mucosal organs and required for lymphocyte homing to mucosal lymphoid tissue (9, 10, 53). Murine endothelial cells can be induced to express high levels of MAdCAM-1 in response to proinflammatory cytokines (8, 9). Recently, it has been shown (28, 57) that the adhesive interactions mediated by MAdCAM-1 and its lymphocyte receptor, αβ7, are involved in leukocyte recruitment to chronic inflammatory diseases of the bowel. MAbs specific for β7 and MAdCAM-1 not only blocked recruitment of lymphocytes to the colitic colon but also reduced the severity of colonic inflammation in severe combined immunodeficient mice reconstituted with CD4+ T cells enriched for the CD45RBhigh subpopulation (42). Our studies indicate that MAdCAM-1 expression in colon is significantly increased in colitic IL-10−/− mice compared with wild-type mice. Together, these results suggest that MAdCAM-1 may also play an important role in initiating and/or maintaining chronic inflammation of colon in IL-10−/− mice and may be a relevant therapeutic target for patients with IBD.

In summary, this study represents the first demonstration that ICAM-1, VCAM-1, and MAdCAM-1 are upregulated in the chronically inflamed cecum and colon of IL-10−/− mice. Furthermore, this study indicates that the transcriptional activation of certain Th1 cytokine(s) or ECAM(s) responsible for the initiation and/or perpetuation of the chronic gut inflammation in this model of colitis is presently under investigation.

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REFERENCES


