Attenuation of colitis in the cotton-top tamarin by anti-alpha 4 integrin monoclonal antibody.

D K Podolsky, … , P Sehgal, M deBeaumont

J Clin Invest. 1993;92(1):372-380. https://doi.org/10.1172/JCI116575.

Research Article

Recent studies have demonstrated the induced expression of endothelial adhesion molecules including E-selectin (also called endothelial leukocyte adhesion molecule-1), vascular cell adhesion molecule and intercellular adhesion molecule in actively involved mucosa of patients with ulcerative colitis and Crohn's disease. Similar induction has been demonstrated in the colon of the Cotton-top tamarin (CTT), a New World primate that experiences a spontaneous acute and chronic colitis resembling ulcerative colitis. To assess the potential importance of leukocyte adhesion as a necessary step in acute colitis, the effect of parenteral mAb directed against adhesion molecules on CTT colitis was evaluated in placebo-controlled blinded trials. Serial administration of either of two anti-E-selectin mAb designated BB11 and EH8 effectively coated endothelial surfaces expressing this vascular adhesion molecule. Although colitis activity was slightly diminished after the 10-d treatment period in CTT receiving either BB11 or EH8, this reduction was not significantly different than that seen in animals given a placebo control when assessed by a previously validated standardized scale of inflammatory activity: mean histologic activity grade 2.2 +/- 0.2 pretreatment vs 1.5 +/- 0.5 posttreatment in group receiving mAb and 2.1 +/- 0.1 pretreatment vs 1.3 +/- 0.5 posttreatment in the placebo group ($P > 0.2$). In contrast, administration of an anti-alpha 4 integrin mAb designated HP1/2 that binds VLA4 (alpha 4 beta 1) and presumably alpha […]

Attenuation of Colitis in the Cotton-top Tamarin by Anti-α4 integrin Monoclonal Antibody

Daniel K. Podolsky,*‡ Roy Lobb,§ Norval King,‡‖ Christopher D. Benjamin,§ Blake Pepinsky,§ Prebhat Sehgal,‡‖ and Michelle deBeaumont*‡

**Gastrointestinal Unit, Massachusetts General Hospital and Department of Medicine, Harvard Medical School; ‡Massachusetts General Hospital/New England Regional Primate Research Center for the Study of Inflammatory Bowel Disease, Boston Massachusetts 02114; §Biogen, Inc., Cambridge, Massachusetts 02142; and ‖New England Regional Primate Research Center, Southboro, Massachusetts 01772*

Abstract

Recent studies have demonstrated the induced expression of endothelial adhesion molecules including E-selectin (also called endothelial leukocyte adhesion molecule-1), vascular cell adhesion molecule and intercellular adhesion molecule in actively involved mucosa of patients with ulcerative colitis and Crohn's disease. Similar induction has been demonstrated in the colon of the Cotton-top tamarin (CTT), a New World primate that experiences a spontaneous acute and chronic colitis resembling ulcerative colitis.

To assess the potential importance of leukocyte adhesion as a necessary step in acute colitis, the effect of parenteral mAb directed against adhesion molecules on CTT colitis was evaluated in placebo-controlled blinded trials. Serial administration of either of two anti-E-selectin mAb designated BB11 and EH8 effectively coated endothelial surfaces expressing this vascular adhesion molecule. Although colitis activity was slightly diminished after the 10-d treatment period in CTT receiving either BB11 or EH8, this reduction was not significantly different than that seen in animals given a placebo control when assessed by a previously validated standardized scale of inflammatory activity: mean histologic activity grade 2.2±0.2 pretreatment vs 1.5±0.5 posttreatment in group receiving mAb and 2.1±0.1 pretreatment vs 1.3±0.5 posttreatment in the placebo group ($P > 0.2$). In contrast, administration of an anti-α4 integrin mAb designated HP1/2 that binds VLA4 ($\alpha4\beta_1$) and presumably $\alpha4\beta_7$ integrins resulted in significant attenuation of acute colitis when compared to both pretreatment activity index ($P = 0.005$) and the placebo control group ($P < 0.01$): mean histologic activity grade 1.6±0.3 pretreatment vs 0.2±0.1 posttreatment in the group receiving HP1/2 and 1.8±0.5 pretreatment and 1.2±0.2 posttreatment in the placebo control group.

These studies using a model of spontaneous colitis in the CTT demonstrate the feasibility of modulation of leukocyte-vascular adhesion and/or other integrin-mediated events possibly including T cell aggregation and T cell-stromal interactions, as well as lymphocyte homing. These results suggest both that these processes are important and possibly essential elements in sustaining acute colitis and that their disruption may result in therapeutic benefit. (*J. Clin. Invest.* 1993. 92: 372–380.) **Key words: adhesion • colitis • Cotton-top tamarin • inflammatory bowel disease**

Introduction

Although the initiating events in the major forms of inflammatory bowel disease (IBD)[1] remain unclear, considerable progress has been made in delineating some of the presumably common, nonspecific inflammatory processes that result in tissue injury and clinical manifestations in association with the periodic increases of inflammatory activity that are the hallmarks of these disorders. Many of these inflammatory factors are found in actively affected tissue from patients with either ulcerative colitis (UC) or Crohn's disease (CD). The various mediators or substances produced by the diverse populations of cellular constituents present at the sites of disease activity include products of arachidonic acid metabolism, both prostaglandins and leukotrienes, a complex mixture of cytokines, and oxygen-free radicals. The relative importance of each of these substances or whether any single one of these mediators is essential to sustaining or amplifying the inflammatory response and injury is not clear. The effect of agents that specifically antagonize individual mediators may provide important insights in this context.

While the absolute dependence of the inflammatory activity on various individual soluble mediators remains uncertain, it is apparent that production of these mediators is intrinsically related to recruitment of inflammatory cells from the vascular compartment to the site of disease activity (1). These include neutrophils, lymphocytes, and monocytes, as well as (to a lesser extent) eosinophils and mast cells. Conversely, disruption of processes responsible for the migration of leukocytes from the vascular space to sites of inflammatory activity might result in attenuation of the generation of inflammatory mediators that are the proximate cause of tissue injury and the manifestations of IBD.

Some of the mechanisms responsible for the targeted migration of leukocytes to various sites of inflammation in the body have recently been delineated (2–7). Specific interactions between proteins present on leukocyte surface membranes and proteins expressed on endothelial surfaces as receptor-counterreceptor pairs appear to be central to these processes. Many of these proteins have been identified and they include members of integrin, selectin, and immunoglobulin protein superfamilies. It is notable that expression of some key adhesion molecules present on the endothelium are specifically induced by cytokines produced at inflammatory foci including interleukin-1, tumor necrosis factor, and interferon (2, 8–11).

Address correspondence to Daniel K. Podolsky, M.D., GI Unit, Jackson 7, Massachusetts General Hospital, 50 Fruit Street, Boston, MA 02114.

Received for publication 30 October 1992 and in revised form 20 January 1993.

J. Clin. Invest.

0021-9738/93/07/0372/09 $2.00
Volume 92, July 1993, 372–380

1. *Abbreviations used in this paper:* CD, Crohn's disease; CTT, Cotton-top tamarin; IBD, inflammatory bowel disease; ICAM1, intracellular adhesion molecule-1; UC, ulcerative colitis; VCAM1, vascular adhesion molecule-1; VLA4, very late antigen-4.

Among the various adhesion molecules identified, endothelial leukocyte adhesion molecule-1 (E-selectin), vascular cell adhesion molecule-1 (VCAM1), and intracellular cell adhesion molecule (ICAM1) on the endothelial surface appear to be particularly important in adhesion of neutrophils, lymphocytes, and monocytes (2–12). E-selectin plays an important role in neutrophil-endothelial adhesion but may also bind monocytes. In addition, E-selectin binds some T cell subsets including memory cells, and may be important in early T cell recruitment (13–16). VCAM1 appears to play a predominant role in lymphocyte and monocyte adhesion to endothelium and ICAM-1 contributes to a wide variety of cellular adhesion events. It should be noted that VCAM1- and ICAM1-mediated interactions may play a role in immune cell activation as well as recruitment. By implication the "counter receptors" on the leukocytes' surfaces are also important and include the putative sialyl Lewis blood group bearing E-selectin ligand, very late antigen-4 (VLA4) and CD11/18 (17–23).

Recent studies in this laboratory and elsewhere have demonstrated the expression of the adhesion molecules E-selectin and ICAM1 at sites of active inflammation in patients with IBD (24, 25). As expected these adhesion molecules are present in both UC and CD. In addition, we have demonstrated the presence of VCAM1 in both inflamed and noninflamed bowel, suggesting it may play a role in the constitutive recruitment of lymphocytes and monocytes to the mucosa. The expression of leukocyte-endothelial adhesion molecules in association with IBD are consistent with the hypothesis that recruitment of leukocytes from the vascular space contribute to the sustained inflammatory response in these disorders. Conversely, it implies that disruption of these processes could attenuate the inflammatory infiltrate. Indeed, one report has indicated that monoclonal antibodies targeted to CD11/18 ameliorated inflammation in a rodent model of colonic inflammation induced by an exogenous agent (26).

The Cotton-top tamarin (CTT) offers a particularly useful model to explore the importance of adhesion molecules in colitis. The CTT is a New World primate that experiences a spontaneous chronic colitis marked by periodic flares of acute inflammation that closely mimics human UC in its histologic and clinical features, as well as its response to pharmacologic agents (27, 28). Previous studies confirmed the induction of E-selectin in association with active colitis in the CTT. Although the lack of reagents suitable for use in this primate precluded assessment of VCAM1, presumably this ligand might also be expressed in the CTT colonic mucosa in a manner similar to that found in man. In this report, we assess the effect of administration of monoclonal antibodies directed against either E-selectin, which contributes to neutrophil-endothelial adhesion or the integrin $\alpha 4$, one of the subunits of VLA4 that contributes to lymphocyte and monocyte adhesion, on the activity of acute flares of CTT colitis. While coating of E-selectin by mAb did not have a significant effect, the anti-$\alpha 4$ mAb led to significant reduction in the acute colonic inflammation.

Methods

Monoclonal antibodies and antisera

BB11 and EH8, two anti–E-selectin mAbs of IgG_{2b} and IgG_1 isotypes, respectively, prepared against the human ligand were demonstrated in earlier studies to recognize the Cotton-top tamarin counterpart (24). Preparation of BB11 has been previously reported (29) while EH8 was prepared by immunization with pure recombinant E-selectin and screening for blockade of neutrophil adhesion to rE-selectin (13). HP1/2, an IgG_1 monoclonal antibody directed against the $\alpha 4$ chain of the human VLA4 heterodimer ($\alpha 4\beta_1$) has been previously described (30) and proven to recognize a cross-reacting determinant on CTT leukocytes by FACS® analysis (Becton Dickinson Immunocytometry Systems, Mountain View, CA) and staining of CTT colonic mucosa. In addition, HP1/2 was proven to block adherence of CTT leukocytes to recombinant soluble VCAM1–coated plates in vitro, as demonstrated below. For in vivo studies mAbs BB11, EH8, and HP1/2 were purified from ascites by protein A chromatography, followed by gel filtration chromatography in endotoxin-free physiologic saline. All mAbs contained < 1 endotoxin unit/mg.

Rabbit anti–human VCAM1 serum (rabbit no. 349) was prepared by immunization of rabbits with recombinant soluble human VCAM1 purified by immunoaffinity chromatography using immobilized anti-VCAM1 mAb 4B9 as described (31). Anti-VCAM1 serum was affinity purified by sequential chromatography on protein A–Sepharose and an affinity resin prepared by immobilization of rsVCAM1 on Affigel (8 mg/ml resin) followed by elution with pH 3.0 buffer, immediate neutralization, and dialysis against PBS. The affinity-purified antiserum completely blocked the binding of human Ramos cells to rsVCAM1–coated plates and binds human, mouse and rat VCAM1 stably expressed in Chinese hamster ovary cells as determined by FACS® analysis.

Evaluation of E-selectin, VCAM1, and α4 integrin expression in CTT

Pinch mucosal biopsies were obtained from rectosigmoid of CTT in parallel with samples obtained for histologic evaluation of inflammation. Tissue samples were placed in OCT compound (Tissue Tek, Elkhart, IN) and frozen sections prepared ($\sim 4\,\mu m$). Immunohistochemistry was performed using a commercially available peroxidase-based technique (Vectastain; Vector Labs, Inc., Burlingame, CA) after incubation with specific first antibody as previously described (24).

Evaluation of serum half-life of anti–E-selectin and anti–α4 integrin mAbs

mAbs BB11, EH8, and HP1/2 were each administered as single intramuscular doses (2 mg/kg) to CTT ($n = 2$ for each antibody) and serum was collected at 0, 1, 2, 6, 12, 24, and 48 h. Levels of mouse Ig were determined by ELISA assay.

Effect of anti–E-selectin and anti–α4 integrin mAbs on CTT colitis

CTT maintained at the New England Regional Primate Research Center were screened for signs of active colitis. Pinch colonic mucosal biopsies obtained after sedation with ketamine (25 mg/kg) by routine techniques were evaluated for active inflammation using a previously validated scoring system (27). CTT with active colitis grades 2+ or 3+ (grading scale range 0–3+) were selected for study to be initiated within 3 wk of the screening biopsy. CTT were rebiopsied at the initiation of the study period. Animals were randomized to receive mAb or placebo (saline) injection.

Anti–E-selectin studies. In separate studies CTT received either BB11 or EH8 ($\sim$ 2 mg/kg) on days 0, 2, 4, 6, and 8; $n = 5$/mAb; placebo controls ($n = 5$ for each mAb trial) were injected intramuscularly with saline on the same schedule. Blood ($\sim$ 0.5 ml) and colonic mucosal biopsies were obtained from mAb and control CTT on days 0, 2, 4, 6, 8, and 10. Serum prepared from peripheral blood was assessed for mouse IgG as noted above. Colonic mucosal biopsies on days 0 and 10 were evaluated for acute colitis activity in a blinded fashion using the validated index noted above. Colonic biopsies were also assessed for the presence of E-selectin using the previously described histochemical technique (24), as well as the presence of mouse IgG. The latter was accomplished by omission of the first antibody in the peroxidase-based staining process.

Anti–α4 integrin studies. CTT received either HP1/2 mAb (2 mg/kg) or saline intramuscular injections ($n = 12$ for each) on days 0, 1, 2,

3, 4, 5, 6, and 7. Colonic mucosal biopsies, as well as peripheral blood, were obtained on days 0, 2, 4, 6, 8, and 10. Colitis activity was assessed in a blinded fashion on biopsies obtained on days 0 and 10. Peripheral blood was used to determine serum mouse IgG levels, as well as complete blood counts.

Results

Expression of vascular adhesion molecules in CTT. In previous studies, E-selectin an inducible leukocyte adhesion molecule expressed on endothelial surfaces was found to be present in association with active colitis in patients with IBD (24). In contrast, VCAM1, a distinct vascular adhesion molecule that binds predominantly lymphocytes and monocytes was present in substantial amounts in association with tissue lymphoid aggregates in colonic mucosa of patients with IBD in a manner independent of active inflammation, as well as normal controls. It should be noted that VCAM1 could also be detected on endothelial surfaces in many of the IBD tissues. Parallel studies demonstrated a similar pattern of expression of E-selectin in colonic mucosa of CTT, a New World monkey that experiences a spontaneous diffuse colitis with close clinical and histopathological similarity to human UC. Thus, anti-E-selectin-stained endothelial surfaces in mucosal biopsies exhibiting signs of active colitis but not those in which the disease process was quiescent. However, it was not possible in those earlier studies to assess the expression of VCAM1 in the primate because of lack of cross-reactivity of the monoclonal reagents prepared against human VCAM1 then available. The development of a conventional antisera against VCAM1 with broad species recognition, as described in Methods, allowed extension of those earlier studies to define VCAM1 expression in the CTT. As illustrated in Fig. 1, VCAM1 is indeed present in the colonic mucosa of CTT as observed in human tissue in earlier studies. As illustrated in Fig. 1, VCAM1 is present in significant amounts in areas of lymphoid aggregates independent of active colitis in a manner similar to that observed in man.

In addition to studies defining the presence of adhesion molecules on the endothelial surface, the expression of one of the cognate counter receptors present on leukocyte surfaces was also examined. Specifically, colonic mucosal biopsies from CTT were assessed for the presence of one of the two subunits that comprise VLA4 by immunohistochemistry using an anti-α4 integrin mAb. As illustrated in Fig. 2, leukocytes, both lymphocytes and monocytes, present in the inflammatory infiltrate in mucosa of CTT with active colitis exhibit the α4 integrin subunit of the VLA4 ligand. Staining was not observed in the absence of the acute cellular inflammatory infiltrate.

Effect of antiadhesion molecule mAbs on CTT colitis. To assess the relative importance of vascular adhesion molecules in sustaining active colitis in the CTT, the effect of parenterally administered mAbs specific for adhesion molecules on active CTT colitis was determined. Initial studies focused on the use of mAbs directed toward E-selectin designated BB11 and EH8. In pilot studies, the individual antibodies were administered as a single intramuscular injection to define the serum half-life for more prolonged therapeutic trials. Administration of these mAbs in concentrations predicted to lead to adequate tissue concentration by previous studies led to extended serum IgG levels in CTT, with a calculated $T_{1/2}$ of 20–24 h for BB11 and EH8. Mucosal biopsies obtained over the course of 48 h after administration of the mAbs led to detectable mouse IgG on the CTT colonic mucosal endothelial surface. Importantly, the presence of IgG was correlated with the lack of ability to detect E-selectin after addition of exogenous anti-E-selectin mAbs to the mucosal biopsies. E-selectin became undetectable by this approach within 6 h of the parenteral administration of the mAb and persisted up to 24 h. However, E-selectin could again be detected to some extent by 24 h and had returned to essentially pretreatment levels by 48 h (Fig. 3).

On the basis of these pilot studies, cohorts of CTT with active colitis as defined by previously validated histological criteria were entered into trials to receive repeated doses of BB11, EH8, or a placebo on alternate days for 8 d. Colonic mucosal biopsies were obtained at the beginning and 2 d after the last mAb injection to ascertain the presence of mAb in the tissue, the expression of E-selectin, and the histological grade of colitis activity. Administration of the anti-E-selectin mAbs led, as anticipated, to demonstrable levels of mouse IgG on endothelial surfaces of CTT receiving these agents. Most importantly, the presence of IgG on endothelial surfaces was again associated with apparent blocking of E-selectin. Despite the apparent coating of E-selectin on endothelial surfaces, neither BB11 or EH8 anti-E-selectin mAb led to a significant improvement in colitis activity when compared to CTT receiving saline placebo (Table I).

The lack of an anti-VCAM1 mAb that recognizes the CTT species precluded direct assessment of the effect of blockade of this endothelial ligand on colitis activity. However, preliminary studies described above demonstrated the presence of α4 integrin, one of the subunits of VLA4 the cognate ligand for VCAM1 on leukocyte surfaces in association with active colitis. The ability of an anti-α4 integrin mAb, HP1/2, to block adhesion of CTT leukocytes to recombinant VCAM1 was confirmed by incubation of isolated leukocytes with HP1/2 before exposure to a surface coated with the recombinant protein (Fig. 4). Adhesion of CTT peripheral blood leukocytes to rsVCAM1 was completely blocked at concentrations of 10 μg/ml.

Subsequently, the effect of the anti-α4 integrin mAb on active CTT colitis was assessed in a manner similar to that used in the study of the anti-E-selectin mAbs. Initial administration of HP1/2 demonstrated a somewhat shorter serum half-life ($T_{1/2}$ = 16 h) than that observed for BB11 and EH8. Initial circulating values after HP1/2 (2 mg/kg) ranged from 10–20 μg falling to the 3–5 μg/ml range at 24 h. This indicated the feasibility of achieving concentrations that affect blocking of CTT VLA4-dependent adhesion events. Because of the shorter half-life, HP1/2 (or saline placebo) was given by daily injection for 8 d and the effect on colitis monitored by histological assessment of colonic mucosal biopsies.

In contrast to the anti-E-selectin mAbs, HP1/2 led to a highly significant improvement in acute colitis when compared to placebo, $P < 0.01$ (Fig. 5 and Table II). Of 12 CTT receiving anti-α4 integrin mAb, all but one showed a reduction in colitis activity at the end of the 10-d study period; eight showed complete quiescence of the acute inflammation. In contrast, six of the CTT receiving placebo showed reduction in colitic activity, and only three had resolution of acute inflammation. Thus, the mean level of acute inflammatory activity was reduced from 1.6±0.3 to 0.2±0.1 after administration of the HP1/2, while activity diminished from 1.8±0.5 to 1.2±0.2 in CTT receiving saline placebo during the same interval ($P < 0.01$). Not surprisingly, no change was observed in histological features that re-

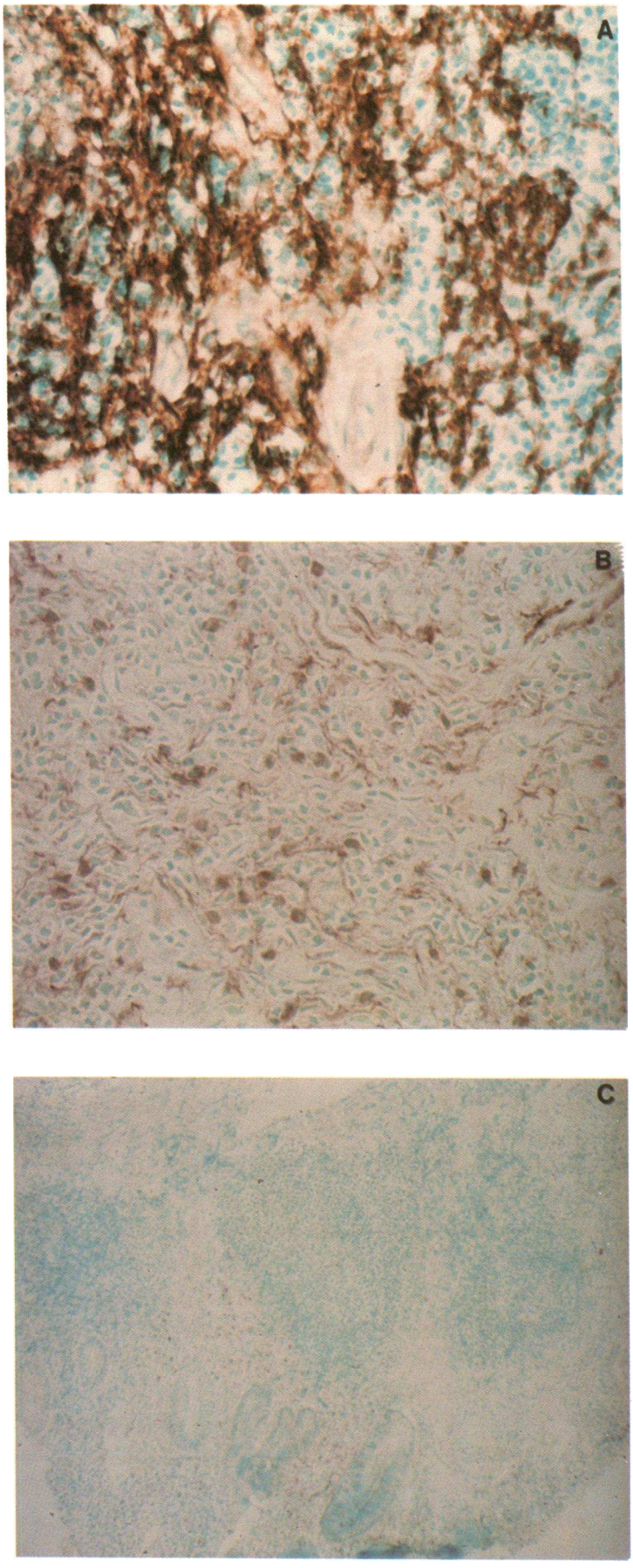

Figure 1. Expression of VCAM1 in colonic mucosa of cotton-top tamarins. Colonic mucosal pinch biopsies were obtained from CTT with active (*A*) and inactive (*B*) colitis. Frozen sections (~ 4 μm) were incubated with affinity-purified rabbit anti-human rsVCAM1. After washing, sections were stained using a peroxidase-based detection system (Vectastain; Vector Labs) and then counterstained with methyl blue. Control sections (*C*) were incubated with a nonimmune rabbit antiserum rather than the anti-rsVCAM1. ×450.

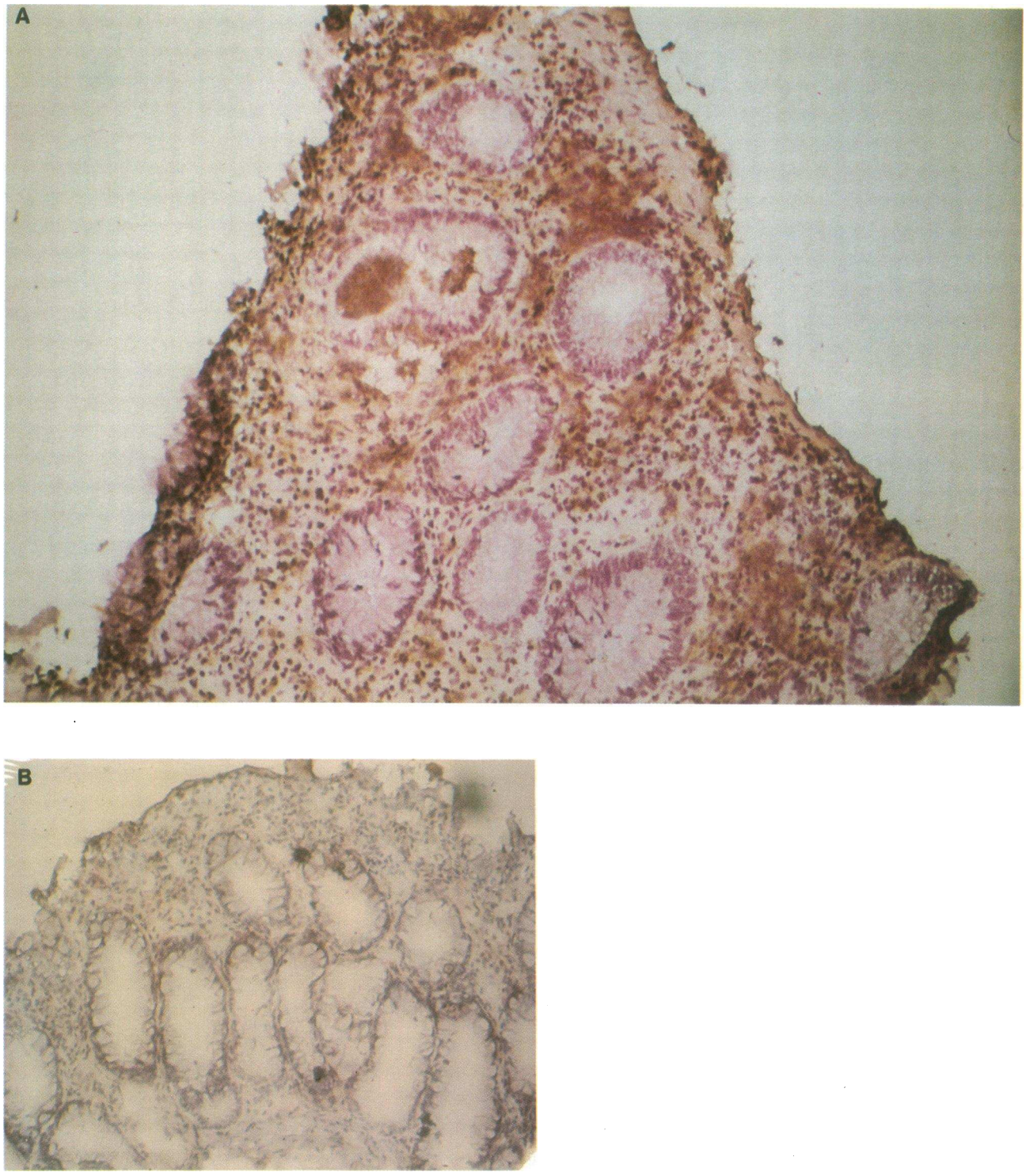

Figure 2. Expression of $\alpha 4$ integrin in colonic mucosa of cotton-top tamarins. Colonic mucosal pinch biopsies were obtained from CTT with active (*A*) and inactive (*B*) colitis. Frozen sections ($\sim$ 4 μm) were incubated with anti-$\alpha 4$ integrin mAb HP1/2. Specifically bound antibody was visualized with the peroxidase-based technique using a conjugated rabbit anti-mouse IgG as noted in legend to Fig. 1 and Methods. Control section (*C*) was incubated with supernatant from the parent NS1 myeloma cell line.

flect chronic mucosal injury using a separate validated scale of chronic injury (24), mean chronic activity remained the same before and after the study period in both CTT receiving HP1/2 and those receiving the placebo. The histological improvement in acute colitis activity was associated with an increase in weight. Mean weight of CTT receiving mAb HP1/2 increased from 394 to 422 g during the treatment period (+5.4%), while CTT receiving saline placebo showed a mean change of −2.7% (409–399 g). Serial leukocyte counts obtained during the treatment period did not show any consistent changes in the periph-

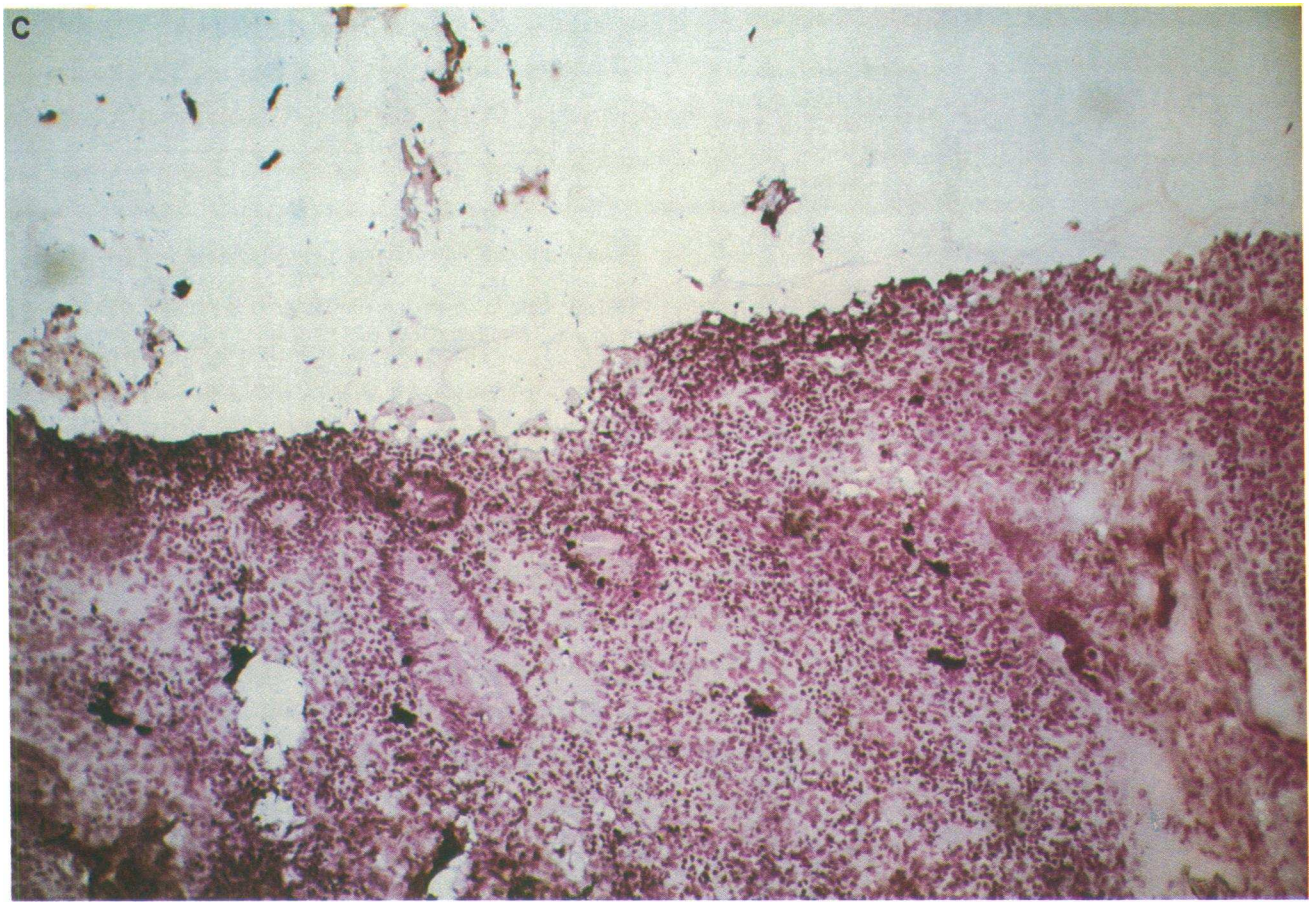

Figure 2 (Continued)

eral blood during the first 2 d of the trial. However, as indicated in Fig. 6 *A*, moderate increases in the peripheral leukocyte counts were observed by day four. Subsequently, levels fell somewhat but remained above initial levels at the conclusion of the study. Parallel determinations showed concomitant reductions in the hematocrit of both CTT receiving mAb and those receiving placebo injection (Fig. 6 *B*). This may reflect the effect of repeated phlebotomy; no signs of overt bleeding diathesis were noted.

Discussion

Recent advances have led to the identification of many of the receptors and counter-receptors present on endothelial and

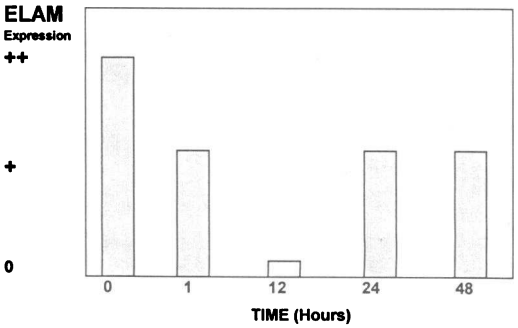

Figure 3. Effect of systemic anti-E-selectin mAbs on detectable colonic mucosal E-selectin. CTTs with active colitis (n = 6 per group) were given BB11 or EH8 anti-E-selectin mAbs intramuscularly (2 mg/kg) or saline placebo by intramuscular injection. Colonic mucosal biopsies were obtained at varying intervals. E-selectin was detected in frozen sections by incubation with BB11 mAb and visualization using a peroxidase-based detection system as described in Methods.

Table I. Effect of anti-E-selectin mAbs on CTT Colitis

Treatment group	Pretreatment	Posttreatment	Δ	
I. mAb-BB11 (n = 5)	2.2±0.2	1.8±0.5	0.4	
				$P > 0.1$
Control (n = 5)	2.0±0	1.4±0.5	0.6	
II. mAb-EH8 (n = 5)	2.2±0.2	1.4±0.5	0.8	
				$P > 0.1$
Control (n = 5)	2.2±0.2	1.2±0.5	1.0	

CTT with active colitis received saline or either of two anti-E-selectin mAbs, BB11, or EH8 (2 mg/kg i.m. on days 0, 2, 4, 6, and 8). Colitis was assessed by blinded evaluation of colonic mucosal biopsies using a standardized grading scale ranging from 3+ (severe) to 0 (inactive). Separate placebo (saline) control group were treated in parallel with each mAb treatment group.

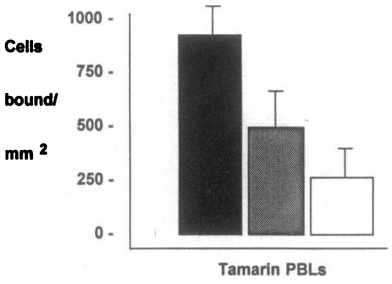

Figure 4. Effect of anti-α4 integrin mAbs on VCAM1-mediated adhesion of CTT leukocytes. Peripheral leukocytes obtained from CTT were incubated with rs VCAM1-coated glass plates alone or in the presence of anti-human VCAM1 mAb 4B9 F(ab)2 or anti-α4 integrin mAb. HP1/2 Adhesion was assessed as the number of adherent cells per square millimeter. ■, rs VCAM; ▩, 4B9 $(Fab')_2$; □, HP1/2.

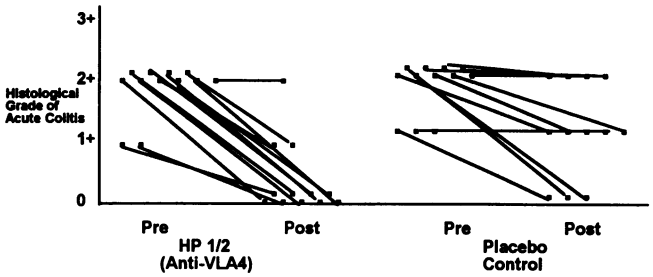

Figure 5. Effect of anti-α4 integrin mAb on CTT Colitis. 24 CTT with acute colitic activity (grade 1+ to 2+ according to a previously validated scale [23]) were randomized (n = 12 per group) to receive either anti-α4 integrin mAb HP1/2 or saline placebo by intramuscular injection lasting for 7 d. Acute colitis was evaluated in a blinded fashion on biopsies obtained on day 0 (*pre*) and day 10 (*post*).

leukocyte surfaces that participate in the adhesion of these cell populations to vascular surfaces at sites of active inflammation. Key endothelial surface proteins including E-selectin and VCAM1 are not constitutively present but are induced by inflammatory cytokines including interleukin-1 and tumor necrosis factor (2, 4, 32–36). Previous studies by ourselves and others have confirmed the presence of E-selectin on endothelial surfaces of colonic mucosa in association with active inflammation in patients with IBD (24, 25). E-selectin appears to be a key ligand recognized by neutrophils and a necessary factor in recruitment of these leukocytes to sites of inflammation. Active colitis in patients with IBD, particularly UC, is marked by a high concentration of neutrophils presumably recruited from the vascular space.

In the studies presented above, the importance of E-selectin–mediated neutrophil adhesion to sustaining active colitis was explored using the CTT as a primate model of colitis closely resembling UC in man. Earlier studies demonstrated that active colitis was associated with endothelial expression of E-selectin in a manner similar to that observed in IBD. Parenteral administration of anti–E-selectin mAbs apparently coated E-selectin present on endothelial surfaces. However, despite the presumed central role of E-selectin–mediated neutrophil adhesion, neither of two anti–E-selectin mAbs studied had a demonstrable effect on CTT colitis. The lack of effect suggests either that E-selectin–dependent neutrophil adherence is not necessary to sustain the inflammatory process or that despite blocking detection of E-selectin by immunohistochemical approaches, functional ligand is still accessible to neutrophils. If, as these results would suggest, E-selectin is not necessary to sustain neutrophil migration into the colonic mucosa, other pathways of neutrophil adhesion must be present in association with the colitis. However, it is also possible that a beneficial effect of E-selectin blockade could have been obscured by complement-mediated inflammation as an indirect effect of immune complex formation on the vessel wall. It is possible that F(ab)2 fragments may be needed to demonstrate the importance of E-selectin modulation in CTT colitis in a manner similar to that observed in a model of lung inflammation in the rat (16).

In addition to E-selectin, earlier studies had demonstrated the expression of another key ligand VCAM1 that participates in adhesion of circulating lymphocytes and monocytes in human colonic mucosa. However, this ligand was found to be present in highest concentrations in mucosal lymphoid aggregates rather than endothelial surfaces. In contrast to E-selectin, VCAM1 expression was found to be present independent of acute inflammation, making determination of its relative importance in the acute inflammatory process difficult. Although

Table II. Effect of anti-α4 Integrin mAb on CTT Colitis

I. Acute activity (mean±SD)

	Pre-Tx	Post-Tx	Δ	
HP 1/2	1.6±0.3	0.2±0.1	1.4	$P < 0.01$
Placebo	1.8±0.5	1.2±0.2	0.6	

II. Chronic activity (mean±SD)

	Pre-Tx	Post-Tx	
HP 1/2	1.6±0.5	1.6±0.5	—
Placebo	2.0±0.5	2.0±0.5	—

CTT with active colitis received saline or anti-α4 integrin mAb HP1/2 (2 mg/kg on days 0, 1, 2, 3, 4, 5, 6, and 7). Colitis was assessed by blinded evaluation of colonic mucosal biopsies using standardized grading scale for acute and chronic inflammation, each ranging 3+ (severe) to 0 (no abnormality). Tx, treatment.

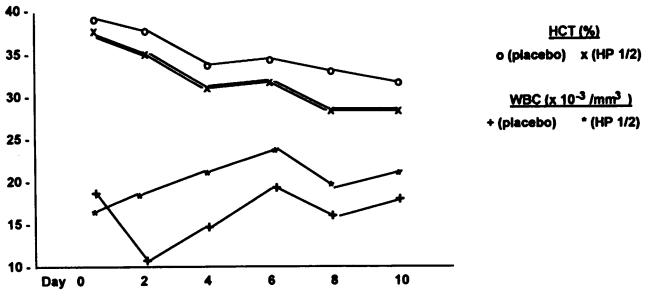

Figure 6. Effect of anti-α4 integrin mAb HP1/2 on peripheral blood counts in CTT. Peripheral leukocyte counts and hematocrit were determined on serial blood samples obtained from CTT receiving anti-α4 integrin mAb HP1/2 (2 mg/kg) every other day as detailed in text.

the reagents available at the time of initial studies precluded assessment of the expression of VCAM1 in CTT, in the present studies the similarity of expression of this adhesion molecule was confirmed with anti-VCAM1 affinity purified from a conventional antiserum. These studies demonstrate the presence of the ligand on endothelial surfaces in both CTT and human colonic mucosa in addition to its expression in mucosal lymphoid aggregates.

Unfortunately, the affinity-purified rabbit anti-VCAM1 reagent was unsuitable to use in studies to determine the effect of blockade of VCAM1-mediated adhesion on colitis in a manner similar to anti-E-selectin mAbs. However, as demonstrated in this report, peripheral leukocytes express $\alpha 4$ integrin, one subunit of the cognate ligand for VCAM1, on leukocyte surfaces, offering another target for assessing these ligands on colitis activity. Thus, an anti-$\alpha 4$ integrin mAb was demonstrated to prevent adhesion of peripheral CTT leukocytes to recombinant VCAM1 indicating its capacity to functionally disrupt the VLA4-VCAM1 mediated process. While anti-E-selectin mAbs had no appreciable effects on CTT colitis, administration of an anti-$\alpha 4$ integrin mAb significantly attenuated acute colitic activity. The ability of anti-$\alpha 4$ integrin mAb to effect an improvement in the colitic activity in CTT provides evidence of the central role that adhesion events mediated through heterodimeric integrins containing this subunit play in this model of IBD. Among the several processes in which the $\alpha 4$ integrin chain is known to participate, it is plausible that the effects of the HP1/2 mAb are at least in part the result of disruption of leukocyte-endothelial adhesion. Thus, the HP1/2 antibody was found to inhibit adherence of CTT leukocytes to VCAM1-coated plates in vitro, a process dependent on VLA4 ($\alpha 4\beta_1$). It is perhaps surprising that blocking this recruitment pathway directed toward lymphocytes and monocytes should have a more profound effect than disruption of the E-selectin-mediated pathway directed towards neutrophils, the more characteristic inflammatory cell hallmark of acute inflammation in IBD. Notably, the anti-$\alpha 4$ integrin led to attenuation of the histologic signs of acute colitis but had no discernable effect on histologic features associated with chronic inflammatory injury in this model.

Although the attenuation of CTT colitis may be most directly attributable to the disruption of leukocyte-vascular adhesion, it should be noted that VLA4 appears to have several functional properties, and it is not possible to know a priori which of these may have been most central to the observed beneficial effect of anti-$\alpha 4$ on colitis (30). Thus, the VCAM/VLA4 pathway may play a role in immune cell activation as well as recruitment (19, 21). VLA4 is known to contribute to T cell–T cell aggregation and, in addition, binds ligands other than VCAM1 including fibronectin (18). It is also possible that the effect of the HP1/2, an mAb directed against the $\alpha 4$ chain of the $\alpha 4\beta_1$ dimer that comprises VLA-4, may in fact reflect binding to $\alpha 4$ associated with $\beta 7$, the ligand for lymphocyte Peyer's patch homing receptors. Recent studies have indicated that anti-$\alpha 4$ not only blocks lymphocyte migration into inflamed tissue in the rat, but also strongly inhibits normal lymphocyte recirculation into mesenteric nodes and intestinal Peyer's patches (37–39). Nonetheless, the reduction in colitis observed suggests an essential role for the ongoing recruitment of lymphocytes and/or monocytes for sustaining active colitic activity. It is clear that the impact on CTT colitis was not a nonspecific effect on concentrations of circulating leukocytes; levels were not decreased but were paradoxically increased within a few days of the initiation of daily injections with the anti-$\alpha 4$ integrin mAb.

As a model of ulcerative colitis, these studies using the CTT underscore the potential therapeutic impact of disrupting the recruitment of inflammatory cells from the vascular space to the site of intestinal inflammation. Further study will be necessary to define the pathways essential to the migration of neutrophils in the context of active colitis. The present findings suggest that the ongoing recruitment of other leukocytes must facilitate the abundant neutrophil representation in the mucosa in this setting. More detailed delineation of the mechanism of anti-$\alpha 4$ inhibition of CTT colitis may provide further insight into the relationship between lymphocyte and monocyte migration and the recruitment of neutrophils in active colitis.

Acknowledgments

The authors thank Gloria Chi-Rosso, Irene Dougas, Kathleen Kimball, and Diane Leone for technical assistance.

These studies supported by grants from the National Institutes of Health (P30DK43351 and R01DK36350 to D. K. Podolsky, and NERPRC 5P51RR00168) and Biogen, Inc.

References

1. Butcher, E. C. 1991. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell.* 67:1033–1036.

2. Bevilacqua, M. P., S. Stengelin, M. A. Gimbrone, Jr., and B. Seed. 1989. Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science (Wash. DC).* 243:1160–1165.

3. Yasuyuki, I., M. S. Singer, C. Fennie, L. A. Lasky, and S. D. Rosen. 1991. Identification of a carbohydrate-based endothelial ligand for a lymphocyte homing receptor. *J. Cell Biol.* 113(5):1213–1221.

4. Osborn, L., C. Hession, R. Tizard, C. Vassallo, S. Luhowskyj, G. Chi-Rosso, and R. Lobb. 1989. Direct expression cloning of vascular cell adhesion. 1. Molecule, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell.* 59:1203–1211.

5. Picker, L. J., A. Warnock, A. R. Burns, C. M. Doerschuk, E. L. Berg, and E. C. Butcher. 1991. The neutrophil selectin LECAM-1 presents carbohydrate ligands to the vascular selectins ELAM-1 and GMP-140. *Cell.* 66(5):921–933.

6. Lobb, R. R., G. Chi-Rosso, D. R. Leone, M. D. Rosa, S. Bixler, B. M. Newman, S. Luhowskyj, C. D. Benjamin, I. G. Dougas, S. E. Goelz et al. 1991. Expression and functional characterization of a soluble form of endothelial-leukocyte adhesion molecule 1. *J. Immunol.* 147:124–129.

7. Jonjic, N., P. Jilek, S. Bernasconi, G. Peri, I. Martin-Padura, S. Cenzuales, E. Dejana, and A. Montovani. 1992. Molecules involved in the adhesion and cytotoxicity of activated monocytes on endothelial cells. *J. Immunol.* 148:2080–2083.

8. Montgomery, K. F., L. Osborn, C. Hession, R. Tizard, D. Goff, C. Vassallo, P. I. Tar, K. Bomsztyk, R. Lobb, and J. M. Harlan. 1991. Activation of endothelial-leukocyte adhesion molecule 1 (ELAM-1) gene transcription. *Med. Sci.* 88:6523–6527.

9. Carlos, T. M., B. R. Schwartz, N. L. Kovach, E. Yee, M. Rosso, L. Osborn, G. Chi-Rosso, B. Newman, R. Lobb, and J. M. Harlan. 1990. Vascular cell adhesion molecule-1 mediates lymphocyte adherence to cytokine-activated cultured human endothelial cells. *Blood.* 76:965–970.

10. Kuijpers, T. W., M. Hoogerwerf, L. J. W. Van der Laan, G. Nagel, C. E. Van der Schoot, F. Grunert, and D. Roos. 1992. CD66 nonspecific cross-reacting antigens are involved in neutrophil adherence to cytokine-activated endothelial cells. *J. Cell Biol.* 118:457–466.

11. Graber, N., T. V. Gopal, D. Wilson, D. L. Beall, T. Polte, and W. Newman. 1990. T cells bind to cytokine-activated endothelial cells via a novel, inducible sialoglycoprotein and endothelial leukocyte adhesion molecule-1. *J. Immunol.* 145:819.

12. Shimizu, Y., W. Newman, T. Venkat Gopal, K. J. Horgan, S. N. Graber, L. Dawson Beall, G. A. Van Seventer, and S. Shaw. 1991. Four molecular pathways of T cell adhesion to endothelial cells: roles of LFA-1, VCAM1, and ELAM-1 and changes in pathway hierarchy under different activation conditions. *J. Cell Biol.* 113:1203–1212.

13. Lobb, R. R., G. Chi-Rosso, D. R. Leone, M. D. Rosa, S. Bixler, B. M. Newman, S. Luhowkyj, C. D. Benjamin, I. G. Dougas, S. E. Goelz, et al. 1991. Expression and functional characterization of a soluble form of endothelial-leukocyte adhesion molecule 1. *J. Immunol.* 147:124–129.

14. Picker, L. J., T. K. Kishimoto, C. W. Smith, R. A. Warnock, and E. C. Butcher. 1991. ELAM-1 is an adhesion molecule for skin-homing T cells. *Nature (Lond.)*. 349:796–799.

15. Shimizu, Y., S. Shaw, N. Graber, T. V. Gopal, K. J. Horgan, G. A. Van Seventer, and W. Newman. 1991. Activation-independent binding of human memory T cells to adhesion molecule ELAM-1. *Nature (Lond.)*. 349:799–802.

16. Mulligan, M. S., J. Varani, M. K. Dame, C. L. Lane, C. W. Smith, D. C. Anderson, and P. A. Ward. 1991. Role of endothelial-leukocyte adhesion molecule 1 (ELAM-1) in neutrophil-mediated lung injury in rats. *J. Clin. Invest.* 88:1396–1406.

17. Phillips, M. L., E. Nudelman, F. C. A. Gaeta, M. Perez, A. K. Singhal, S. I. Hakomori, and J. C. Paulson. 1990. ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, Sialyl-Le^x. *Science (Wash. DC)*. 250:1130–1132.

18. Elices, M. J., L. Osborn, Y. Takada, C. Crouse, S. Luhowskyj, M. E. Hemler, and R. R. Lobb. 1990. VCAM1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell.* 60:577–584.

19. Pulido, R., M. J. Elices, M. R. Campanero, L. Osborn, S. Schiffer, A. Garcia-Pardo, R. Lobb, M. E. Hemler, and F. Sanchez-Madrid. 1991. Functional evidence for three distinct and independently inhibitable adhesion activities mediated by the human integrin VLA-4. *J. Biol. Chem.* 266:10241–10245.

20. Tyrrell, D., P. James, N. Rao, C. Foxall, S. Abbas, F. Dasgupta, M. Nashed, A. Hasegawa, M. Kiso, D. Asa, J. Kidd, and B. K. Brandley. 1991. Structural requirements for the carbohydrate ligand of E-selectin. *Biochemistry.* 88:10372–10376.

21. Van Dinther-Janssen, A. C. H. M., E. Horst, G. Koopman, W. Newmann, R. J. Scheper, C. J. L. M. Meijer, and S. T. Pals. 1991. The VLA-4/VCAM-1 pathway is involved in lympthocyte adhesion to endothelium in rheumatoid synovium. *J. Immunol.* 147:4207–4210.

22. Berg, E. L., M. K. Robinson, O. Mansson, E. C. Butcher, and J. L. Magnani. 1991. A carbohydrate domain common to both Sialyl Le^a and Sialyl Le^x is recognized by the endothelial cell leukocyte adhesion molecule ELAM-1. *J. Biol. Chem.* 266:14869–14872.

23. Berg, E. L., J. Magnani, R. A. Warnock, M. K. Robinson, and E. C. Butcher. 1992. Comparison of L-selectin and E-selectin ligand specificities: the L-selectin can bind the E-selectin ligands Sialyl Le$_x$ and Sialyl Le^a. *Biochem. Biophys. Res. Commun.* 184:1048–1055.

24. Koizumi, M., N. King, R. Lobb, C. Benjamin, and D. K. Podolsky. 1992. Expression of vascular adhesion molecules in inflammatory bowel disease. *Gastroenterology.* 103:840–847.

25. Malizia, G., A. Calabrese, M. Cottone, M. Raimondo, L. K. Trejdosiewicz, C. J. Smart, L. Oliva, and L. Pagliaro. 1991. Expression of leukocyte adhesion molecules by mucosal mononuclear phagocytes in inflammatory bowel disease. *Gastroenterology.* 100:150–159.

26. Wallace, J. L., A. Higa, G. W. McKnight, and D. E. MacIntyre. 1992. Prevention and reversal of experimental colitis by a monoclonal antibody which inhibits leukocyte adherence. *Inflammation.* 16:343–354.

27. Madara, J. L., D. K. Podolsky, N. W. King, P. K. Seghal, R. Moore, and H. S. Winter. 1985. Characterization of spontaneous colitis in cotton-top tamarins (*Saguinus oedipus*) and its response to sulfasalazine. *Gastroenterology.* 88:13–9.

28. Podolsky, D. K., J. L. Madara, N. King, P. Sehgal, R. Moore, and H. S. Winter. 1985. Colonic mucin composition in primates. Selective alterations associated with spontaneous colitis in the Cotton-top tamarin. *Gastroenterology.* 88:20–25.

29. Benjamin, C., I. Dougas, G. Chi-Rosso, S. Luhowskyj, M. Rosa, B. Newman, L. Osborn, C. Bassallo, C. Hession, S. Goelz, K. McCarthy, and R. Lobb. 1990. A blocking monoclonal antibody to endothelial-leukocyte adhesion molecule-1 (E-selectin). *Biochem. Biophys. Res. Commun.* 171:348–353.

30. Pulido, R., M. J. Elices, M. R. Campanero, L. Osborn, S. Schiffer, A. Garcia-Pardo, R. Lobb, M. E. Hemler, and F. Sanchez-Madrid. 1991. Functional evidence for three distinct and independently inhibitable adhesion activities mediated by the human integrin VLA-4. *J. Biol. Chem.* 266:10241–5.

31. Lobb, R., G. Chi-Rosso, D. Leone, M. Rosa, B. Newman, S. Luhowskyj, L. Osborn, S. Schiffer, C. Benjamin, I. Dougas, C. Hession, and P. Chow. 1991. Expression and functional characterization of a soluble form of vascular cell adhesion molecule 1 (VCAM-1). *Biochem. Biophys. Res. Commun.* 178:1498–1504.

32. Kansas, G. S., O. Spertini, L. M. Stoolman, and T. F. Tedder. 1991. Molecular mapping of functional domains of the leukocyte receptor for endothelium, LAM-1. *J. Cell Biol.* 114:351–358.

33. Yamada, K. M. 1991. Adhesive recognition sequences. *J. Biol. Chem.* 266:12809–12812.

34. Hession, C. L., Osborn, D. Goff, G. Chi-Rosso, C. Vassallo, M. Pasek, C. Pittack, R. Tizard, S. Goelz, K. McCarthy, S. Hopple, and R. Lobb. 1990. Endothelial leukocyte adhesion molecule 1: direct expression cloning and functional interactions. *Cell Biol.* 87:1673–1677.

35. Carlos, T. M., B. R. Schwartz, N. L. Kovach, E. Yee, M. Rosso, L. Osborn, G. Chi-Rosso, B. Newman, R. Lobb, and J. M. Harlan. 1990. Vascular cell adhesion molecule-1 mediates lymphocyte adherence to cytokine-activated cultured human endothelial cells. *Blood.* 76:965–970.

36. Postigo, A. A., R. Garcia-Vicuna, F. Diaz-Gonzalez, A. G. Arroyo, M. O. DeLandazuri, G. Chi-Rosso, R. R. Lobb, A. Laffon, and F. Sanchez-Madrid. 1992. Increased binding of synovial T lymphocytes from rheumatoid arthritis to endothelial-leukocyte adhesion molecule-1 (ELAM-1) and vascular cell adhesion molecule-1 (VCAM-1). *J. Immunol.* 89:1445–1452.

37. Issekutz, T. B. 1991. Inhibition of in vivo lymphocyte migration to inflammation and homing to lymphoid tissues by the TA-2 monoclonal antibody: a likely role for VLA-4 in vivo. *J. Immunol.* 147:4178–4184.

38. Rüegg, C., A. A. Postigo, E. E. Sikorski, E. C. Butcher, R. Pytela, and D. J. Erle. 1992. Role of integrin $\alpha4\beta7/\alpha4\beta$P in lymphocyte adherence to fibronectin and VCAM-1 and in homotypic cell clustering. *J. Cell Biol.* 117:179–189.

39. Holzmann, B., and I. L. Weissman. 1989. Integrin molecules involved in lymphocyte homing to Peyer's patches. *Immunol. Rev.* 108:45–62.