Antibody Blockade of ICAM-1 and VCAM-1 Ameliorates Inflammation in the SAMP-1/Yit Adoptive Transfer Model of Crohn's Disease in Mice

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Background & Aims: Integrins (α_4 and β_2) and their endothelial ligands vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) play key roles in leukocyte recruitment to areas of inflammation. ICAM-1 and VCAM-1 are expressed in inflamed intestinal tissues. This study investigates a possible causative role of adhesion molecules ICAM-1, VCAM-1, and α_4 integrins in mediating the inflammatory response in a murine model of Crohn's disease (CD). *Methods:* $CD4^+$ mesenteric lymph node cells from SAMP-1/Yit donor mice were adoptively transferred into major histocompatibility complex–matched severe combined immunodeficiency disease mice. Six weeks later, these mice were left untreated or treated for 3 days with monoclonal antibodies (mAbs) to ICAM-1, VCAM-1, or both, and α_4, or both ICAM-1 and α_4, dexamethasone, or nonblocking isotype control antibodies. On day 4 after treatment, tissues were investigated for expression of ICAM-1, VCAM-1, and for severity of inflammation using a semiquantitative inflammatory score. Dexamethasone treatment resolved all measures of intestinal inflammation. *Results:* Blocking either ICAM-1, VCAM-1, or α_4 integrins had no significant beneficial effect. However, blocking ICAM-1 and α_4, or blocking ICAM-1 and VCAM-1, showed a 70% resolution of the active inflammation, but not chronic inflammation. *Conclusions:* These findings suggest that blocking ICAM-1 and VCAM-1 may have therapeutic benefit for the acute inflammatory component of Crohn's disease.

Crohn's disease (CD) in humans affects nearly one million Americans and is increasing in incidence. Clinically, it is marked by relapsing episodes of abdominal pain, diarrhea, weight loss, and may progress to fistula formation and bowel obstruction. Transmural inflammation, fistula, and granuloma formation that can be randomly distributed along the gastrointestinal tract from the mouth to the anus characterize the disease, although the terminal ileum is the most common location.[1] The etiology of CD remains unknown. Genetic, immunologic, and environmental factors have been proposed and investigated.[2–5] Current treatment regimens are based on immunosuppression with glucocorticoids, azathioprine, methotrexate, and cyclosporin A.[6] Recent clinical reports have noted improvement in symptoms and in healing of fistula tracts with the use of tumor necrosis factor (TNF) antibodies.[7,8] These findings suggest that intervening with the acute inflammatory response may be beneficial in the treatment of CD.

Leukocyte and endothelial adhesion molecules play a key role in mediating the recruitment of leukocytes to areas of inflammation.[9,10] Intercellular adhesion molecule-1 (ICAM-1) is an immunoglobulin-like adhesion molecule expressed on endothelial cells, fibroblasts, and many other cell types[11,12] and is up-regulated in inflammation.[11–13] Leukocyte ligands for ICAM-1 include LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18). ICAM-1 has been implicated in basal trafficking of neutrophils and lymphocytes,[14] and in the recruitment of inflammatory cells to some,[15] but not all,[16] models of inflammation. In addition, ICAM-1 antibody treatment has been effective in rheumatoid arthritis, where sustained improvement in disease has been effected after a 2- or 5-day course of treatment. The precise mechanism for this sustained improvement in disease remains to be elucidated, but other investigators have proposed induction of T-cell hyporesponsiveness.[17–20] We hypothesized that interfering with ICAM-1 function may reduce the inflammatory infiltrate in our murine model of Crohn's disease. Vascular cell adhesion molecule-1 (VCAM-1) also belongs to the immunoglobulin-like family of ad-

Abbreviations used in this paper: α_4, alpha 4 integrin; ICAM-1, intercellular adhesion molecule-1; mAb, monoclonal antibody; SAMP-1, senescence accelerated mouse P-1; SCID, severe combined immune deficiency; TNF, tumor necrosis factor; VCAM-1, vascular cell adhesion molecule-1.

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Table 1. Histologic Scoring System

Feature	Grade	Description
Active inflammation	0.5	Scattered PMNs in lamina propria, <5 intercryptal spaces contiguously involved in an area
	1	Scattered PMNs in lamina propria, ±focal invasion of epithelium, 5 or more intercryptal spaces contiguously involved. No crypt abscesses
	2	Increase of PMNs within lamina propria and/or submucosa, with focal aggregates of 5 or more cells. Relative increase of PMNs within epithelium, ± crypt abscesses
	3	Confluent areas of crypt abscesses and/or mucosal erosion/ulceration accompanied by an active inflammatory infiltrate
Chronic inflammation	0.5	Minimal increase of mononuclear inflammatory cells in lamina propria, based primarily on increased cell number below base of crypts. Less than 5 intercryptal spaces involved in an area. Intercryptal space is not significantly increased over normal
	1	Minimal increase of mononuclear inflammatory cells in lamina propria, based primarily on increased cell number below base of crypts. An area of 5 intercryptal regions or more is involved in an area. Intercryptal space is not significantly increased over normal
	2	Increase of chronic inflammation in lamina propria such that crypts are separated by ≥½ but <1 crypt diameters or crypt bases are separated from the muscularis mucosa by ≥½ but <1 crypt diameters
	3	Increase of chronic inflammation in lamina propria such that crypts are separated by ≥1 crypt diameters or crypt bases are separated from the muscularis mucosa by ≥1 crypt diameters
Villus architecture	1	Villus height decreased by <⅓ and/or villus width increased <50% of normal
	2	Villus height decreased ⅓ to ⅔ and/or villus width increased to >50% of normal
	3	Villi absent, or height reduced to <⅔ of normal

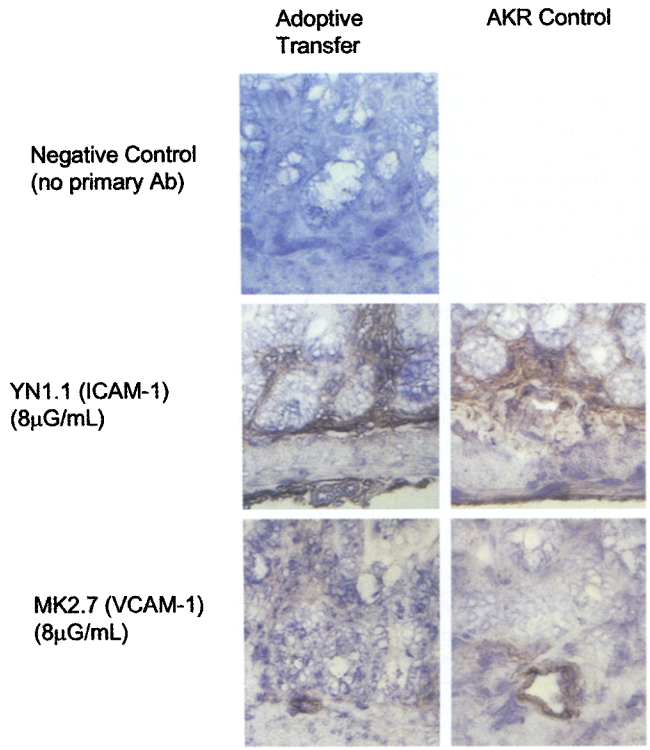
Cross-sectional area	Numerical score
<1%	0.5
1%–25%	1
26%–50%	2
51%–75%	3
76%–100%	4

NOTE. If various grades of the specific histologic feature are present, then the most abundant grade is used as the base grade. If the minor histologic component is a higher grade, then the base grade is modified by adding 0.5. Example, a moderately inflamed intestine (grade 2) has only a single area of erosion (grade 3), then the overall grade for active inflammation is 2.5. The final histologic score for these 3 features is determined by multiplying the histologic grade by the numerical assessment of area involved (example: active inflammation score of an animal with a moderately active [grade 2] enteritis involving 30% of the examined small intestine is $2 \times 2 = 4$).

hesion molecules and is inducible on endothelial cells and vascular smooth muscle cells with a slower time course than ICAM-1, peaking at 24 hours in some models.[21] VCAM-1 binds $\alpha_4\beta_1$ integrin (CD49d/CD29) on lymphocytes, eosinophils, and monocytes and $\alpha_d\beta_2$ (CD11d/CD18) on lymphocytes. VCAM-1 is associated with chronic types of inflammation, like asthma[22] or atherosclerosis.[23] Inflammatory bowel disease (IBD) can be transferred by $CD4^+$ lymphocyte,[24,25] most of which express α_4 integrins. Furthermore, previous animal studies with chemical irritant–induced colitis have shown that blockade of either ICAM-1 or VCAM-1 can dramatically reduce the leukocyte adherence and disease activity index in the inflamed colon.[26,27] Therefore, we hypothesized that our model of CD, induced by adoptive transfer of unfractionated $CD4^+$ T cells into SCID mice, may respond to blockade of VCAM-1 or α_4 integrins. We reasoned that these interventions could limit recirculation of pathogenic lymphocytes to the intestinal tissues and may limit access of monocytes, eosinophils, and neutrophils. Under inflammatory conditions, neutrophils can also express $\alpha_4\beta_1$,[28,29] so that anti-α_4 treatment may also reduce neutrophil recruitment.

The role for these molecules in mediating the inflammatory process appear to be tissue-specific, and to some degree, specific to the origin of the inflammatory stimulus. Recent studies in human CD have shown an association between expression of adhesion molecules and disease activity in IBD.[30,31] Some adhesion molecules are proteolytically removed from the cell surface and can be found in the serum, where their concentration is increased in IBD patients.[32,33] These studies have generated an interest in the role of adhesion molecules as markers and potentially causative factors and targets for therapeutic interventions in IBD.

The study of IBD, and specifically CD, has been hampered by the lack of an animal model that closely mimics the human form of the disease. Several models of caustic exposure of the gastrointestinal tract to various chemicals have been developed,[34–36] although their relevance to human CD is uncertain. Recently, murine models have been identified to spontaneously develop



intestinal inflammation.[36–39] Of these, only the senescence accelerated mouse P-1 (SAMP-1)/Yit model has been shown to have primarily small bowel inflammation that resembles human CD.[30] The SAMP-1/Yit mouse is an inbred strain of senescence-accelerated mouse, which spontaneously develops transmural granulomatous inflammatory ileitis closely mimicking the findings associated with CD in humans.[37]

Recently, our group found that the Crohn's-like disease can be transferred to mice with severe combined immune deficiency (SCID) by adoptive transfer of unfractionated $CD4^+$ T cells.[25] Adoptive transfer leads to a predictable and reproducible inflammation of the terminal ileum at 6 weeks after transfer. In the present study, we use this model because it produces homogeneous

Figure 1. Adhesion molecule expression in the adoptive transfer model. Increased staining for ICAM-1 in SCID mice reconstituted with 10^6 $CD4^+$ T cells from SAMP-1/Yit mice (*left column*), compared with controls receiving AKR $CD4^+$ T cells (*right column*). Negative control, no primary antibody.

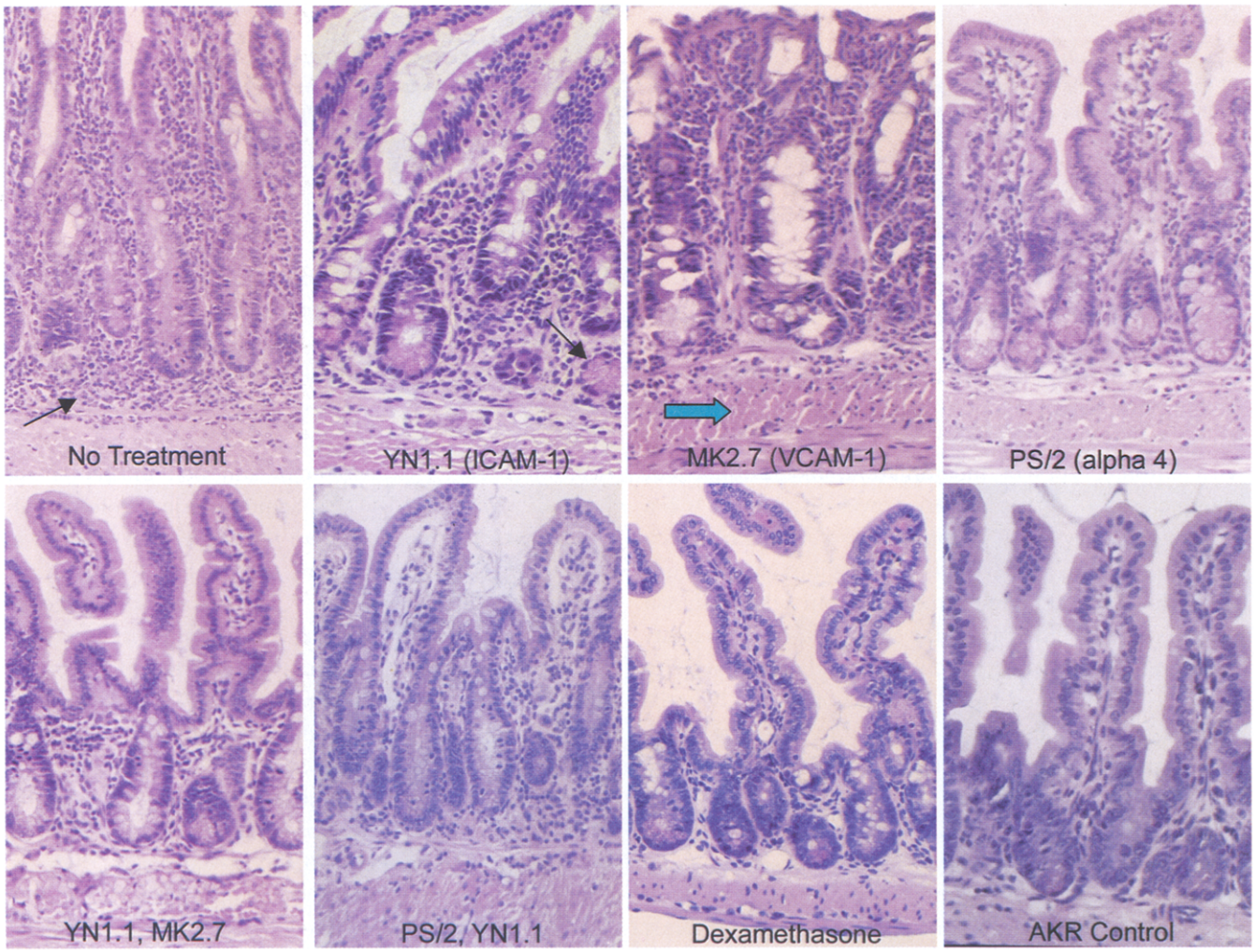


Figure 2.

groups of mice with exactly timed disease onset to investigate our novel treatment schemes. Our experiments test the hypothesis that interventions blocking α4 integrins or VCAM-1 may affect lymphocyte homing and recirculation to the gut.[40,41] We reasoned that if anti-α_4, and/or anti-VCAM-1 treatment was effective, this would indicate that α_4^+ T cells, probably of a Th1 phenotype,[42,43] continuously gain access to the inflamed section of the intestine and contribute to disease progression. In addition, we aimed at ICAM-1, which is involved in trafficking of lymphocytes, neutrophils, monocytes, and eosinophils. When we observed a beneficial effect of blocking both ICAM-1 and VCAM-1, we further tested whether a similar effect could be obtained by targeting the leukocyte ligands of VCAM-1, α_4 integrins.

Materials and Methods

Adoptive Transfer

Mesenteric lymph nodes were obtained from anesthetized SAMP-1/Yit mice (30–50 weeks old, maintained in a colony at the University of Virginia) and lymphocytes were rendered into a single-cell suspension as described by Kosiewicz[25] and positively selected for $CD4^+$ by magnetic beads (Miltenyi Biotech, CA). $CD4^+$ lymphocytes were obtained, counted, and injected at 1×10^6 $CD4^+$ lymphocytes per mouse intraperitoneally (IP) into 6–8 week old C3HeJ-SCID mice (Jackson Laboratories, Bar Harbor, ME). The mice were housed in a barrier facility and were fed irradiated, standard mouse chow. After 6 weeks, the adoptively transferred mice showed a disease similar to that of the original SAMP-1/Yit mice with moderate to severe lymphocyte infiltration and villus blunting. Some mice received $CD4^+$ T cells from AKR mice (n = 6, Jackson Laboratories) and failed to develop inflammation.

Treatment Interventions

A 3-day course of therapy was administered during the last 3 days of the 6-week posttransfer period. Monoclonal antibodies (mAbs; 100 μg each) YN-1 (rat IgG2b directed to mouse ICAM-1, American Type Culture Collection [ATCC], Manassas, VA, n = 12), MK2.7 (rat IgG1 directed to mouse VCAM-1, n = 11), PS/2 (rat IgG2b directed to mouse α_4, ATCC, n = 17), combinations of YN-1 and MK2.7, ATCC, (n = 12), or a combination of YN-1 and PS/2 (n = 12) were injected IP daily for 3 days. These mAbs were produced and purified form hybridoma supernatants at the University of Virginia biomolecular core facility. Separate groups were treated with nonblocking isotype control mAbs (IgG1 n = 10, $IgG2_b$ n = 16; Pharmingen, San Diego, CA) or dexamethasone (100 μg IP for 3 days, American Regent Laboratories, Shirley, NY; n = 19) as a positive control, or left untreated as a negative control (n = 14). These treatment schemes were also applied to groups (n = 8 per group) of 40-week-old SAMP-1/Yit mice.

The adoptively transferred SCID mice or SAMP-1/Yit mice were euthanized after the 3-day course of treatment. The small bowel was harvested. The terminal ileum and 15-cm proximal was opened and rolled longitudinally for histologic scoring of inflammatory disease. The bowel was fixed in Bouins solution, embedded in paraffin, sectioned, and processed with hematoxylin and eosin staining. A standard histologic scoring system was developed and used to evaluate inflammatory index (Table 1). Scores were given for 3 histologic changes: (1) active inflammation (neutrophil infiltration of tissue); (2) chronic inflammation (lymphocytes, plasma cells, and macrophages in the mucosa and submucosa); and (3) villus distortion (flattening and/or widening of normal mucosal villous architecture). Scores ranged from 0 to 3 with 0 showing normal histology and 1–3 showing incremental severity of histologic changes (Table 1). Scoring was conducted by an independent histopathologist (C.A.M.), blinded to the treatment groups. In addition, the presence or absence of granuloma formation (aggregates of epithelioid histiocytes), multinucleated histiocytic giant cell formation and hypertrophy of the intestinal muscular layer were noted.

Immunohistochemistry

Ileum of adoptively transferred SCID mice was obtained at 6 weeks after adoptive transfer. Ileal samples were snap frozen and sections (5 μm) were cut on a cryostat (Microm HM505N, Walldorf, Germany). Immunostaining was conducted using overnight incubation with primary mAb (YN-1 against ICAM-1, or MK2.7 against VCAM-1) following endogenous peroxidase quenching with MetOH and 30% H_2O_2. Nonspecific binding was reduced using fish-skin gelatin oil and normal goat serum (Sigma, St. Louis, MO). Secondary staining was conducted with goat anti-rabbit antibody, avidin, biotin peroxidase (Vector, Burlingame, CA). Untreated AKR mice, following the same immunostaining protocol, served as negative controls.

Statistics

Groups were compared with control (no treatment) and to Isotype control antibody (Isotype) by 1-way analysis of variance (ANOVA) with Dunn's or Tukey's modification using

Figure 2. Representative histologic sections in the adoptive transfer model. The single antibody treatment groups (YN1.1 n = 12, MK2.7 n = 11, and PS/2 n = 17) show an increased lymphocytic infiltrate (*upward arrow*, not seen in all), with granuloma formation (*downward arrow*, not seen in all). The combined treatment groups, ICAM-1/VCAM-1 (YN1.1, MK2.7, n = 12), and ICAM-1/α_4 (YN1.1, PS/2, n = 12) show marked resolution of leukocyte infiltrate, and slight improvement in villus blunting, granuloma formation, and muscular hypertrophy. The AKR control and dexamethasone (n = 19) groups, in comparison, reveal no inflammation and no villus blunting.

SPSS statistical software. Data are expressed as mean and standard error of the mean, with significance accepted at the level of $P \leq 0.05$.

Results

Expression of Vascular Immunoglobulin Adhesion Molecules

Immunostaining revealed increased staining for ICAM-1 in the mice adoptively transferred with $CD4^+$ T cells from SAMP-1/Yit mice compared with SCID receiving $CD4^+$ T cells from AKR mice (Figure 1). This staining was present in vascular cells, and the lamina propria. Staining for VCAM-1 was restricted to vascular structures in mice adoptively transferred with SAMP-1/Yit and low in SCID mice adoptively transferred with AKR $CD4^+$ T cells.

Inflammation and Villus Blunting in Adoptively Transferred Mice

Hematoxylin and eosin staining of the ileal samples was used to show the degree of inflammation in the various treatment groups (Figure 2). SCID mice receiving 10^6 $CD4^+$ T cells from SAMP-1/Yit showed more severe inflammation with lymphocyte infiltration, granuloma formation, villus distortion, and muscular hypertrophy. SCID mice receiving $CD4^+$ T cells from AKR showed almost no inflammation and no villus blunting. Their histologic scores were not significantly different from SCID mice receiving no T cells (data not shown).

Treatment With mAbs to ICAM-1, VCAM-1, and α_4 Integrin

The group treated with mAb to ICAM-1 alone showed a trend toward improvement in active inflammation and villus blunting, but these do not reach statistical significance. The lymphocyte infiltration persisted, as did villus distortion and muscular hypertrophy (Figure 2). Treatment with mAb to VCAM-1 or α_4 integrin alone failed to show resolution of active or chronic inflammation or villus distortion. The group treated with mAb to both ICAM-1/VCAM-1 showed slight improvement in granuloma formation and inflammatory cell infiltrate, and a trend toward ameliorated chronic inflammation, but muscular hypertrophy persisted, as did villus distortion. The ICAM-1/α_4 group showed significant resolution of active inflammatory cell infiltration and some improvement in chronic inflammation, but still muscular hypertrophy. The positive control groups receiving dexamethasone showed complete resolution of the lymphocytic infiltrate, villus distortion, granuloma formation, and muscular hypertrophy to near control levels (Figure 2).

Treatment of SAMP-1/Yit Mice

To test whether anti-adhesion molecule treatment would also be effective in ameliorating inflammation in SAMP-1/Yit mice, groups of 8 mice (40 weeks old) were treated with mAbs to ICAM-1, VCAM-1, α_4 integrin or combination of ICAM-1 and VCAM-1, or ICAM-1 and α_4 (100 μg each for 3 days). Dexamethasone was used as a positive control (100 μg daily for 3 days). The baseline inflammatory scores in the SAMP-1/Yit mice were not different from the AT groups, indicating a similar severity of inflammatory disease. Although dexamethasone showed resolution of active and chronic inflammation and villus distortion, treatment with mAbs did not result in significant improvement in any of the histologic scores (Figure 3). These findings suggest that the inflammatory process in the SAMP-1/Yit model is mediated by different or additional processes than that in the adoptive transfer model.

Discussion

In a novel murine model of human Crohn's disease, we show that adoptive transfer of unfractionated $CD4^+$ T cells from SAMP-1/Yit mice into SCID recipients induces expression of the endothelial adhesion molecules ICAM-1 and VCAM-1. Blocking both ICAM-1 and VCAM-1, but not either one alone, almost completely resolves the acute inflammatory infiltrate. Previous experiments in this[25] and other[46] models have shown that disease-causing T cells are of a Th1-like phenotype, producing interferon-γ (IFN-γ) and TNF-α, but not IL-4 or IL-10.

The requirement for blockade of both ICAM-1 and VCAM-1 may be related to the ability of activated T cells to use either adhesion pathway for access to inflamed tissues.[47] Such a redundancy of pathways has previously been shown in other models of inflammation,[48] and has also been shown for other sets of adhesion molecules, for example P-selectin and E-selectin in neutrophil recruitment.[49] During the 3-day treatment scheme, any neutrophils present in the intestinal tissue at the beginning of treatment would likely have been eliminated by apoptosis, despite the previously demonstrated delay in neutrophil apoptosis in human inflammatory bowel disease (IBD) tissues.[50–52] The almost complete resolution of the acute inflammatory response suggests that new neutrophils are not recruited in sufficient numbers to maintain inflammation under this treatment scheme. This may be caused directly by the role of both α_4 and VCAM-1 in neutrophil recruitment as recently shown in acute and chronic inflammatory models.[53,54] However, the lack of

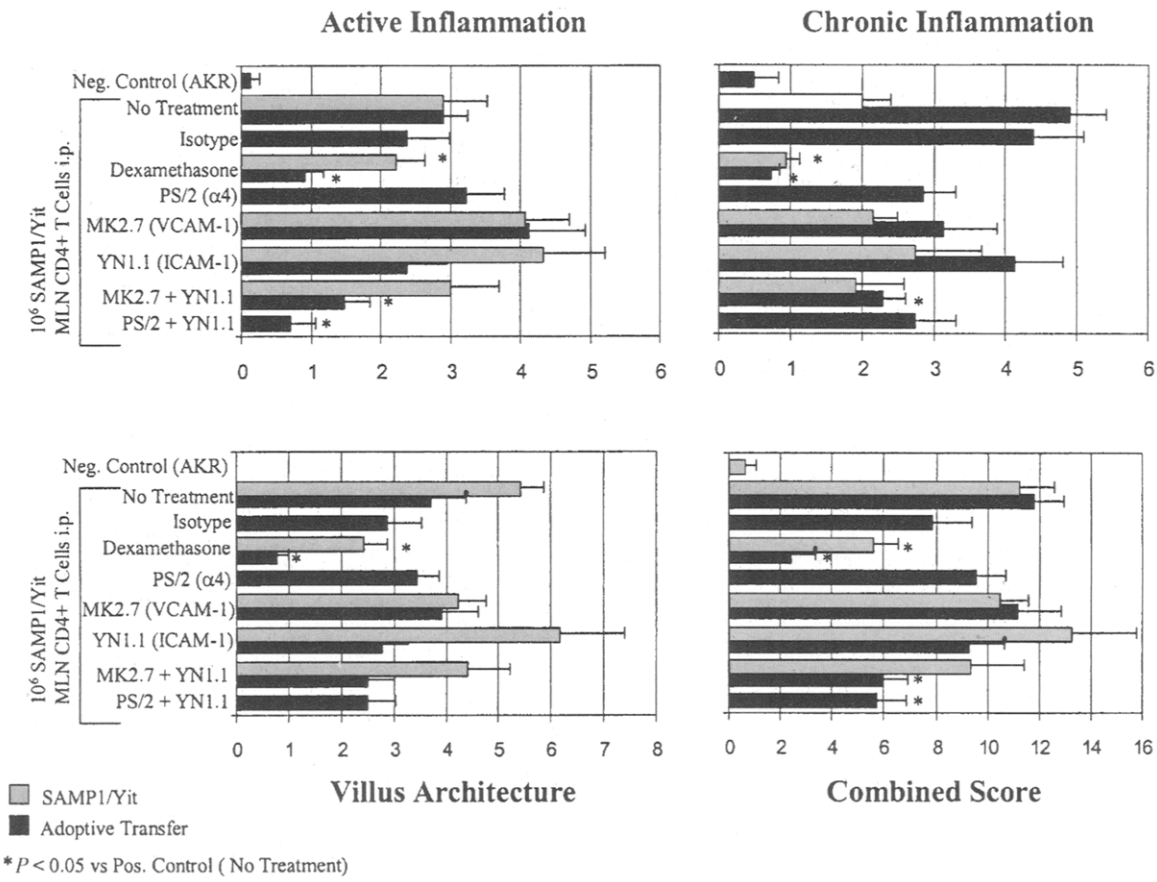


Figure 3. Histologic scores for active inflammation, chronic inflammation, villus architecture as defined in Table 1. Data are reported as mean, with standard error of the mean, significance is accepted at $P < 0.05$. Significant reduction of active, chronic inflammation, and combined scores ($P < 0.05$) in the AT model only by blocking ICAM-1 and VCAM-1 for 3 days before analysis, significant reduction of active inflammation and combined score in the AT model by blocking α_4 integrin and ICAM-1 ($P < 0.05$). No effect on villus architecture. Dexamethasone ameliorated all aspects of inflammation in both the AT model and in the SAMP-1/Yit mice ($P < 0.05$).

effect of anti-ICAM-1 treatment alone suggests that this is not necessarily a direct effect. It is also possible that reduced neutrophil accumulation as seen by histology may be secondary to altered or reduced lymphocyte trafficking. In addition, longer treatment schema may further delineate the role of blockade of individual adhesion molecules. Further experiments will be needed to delineate the cytokines and chemokines produced by the disease-conferring T cells that drive the inflammatory response.

The notion that interfering with T-cell trafficking can curb the inflammation in this model of Crohn's disease is supported by our finding that blocking α_4 integrins, the lymphocyte ligand for VCAM-1, is at least as efficient as blocking VCAM-1 when used in combination with ICAM-1 blockade. This finding suggests a mechanistic link between the beneficial therapeutic effects seen in our study and the etiopathology of the disease. However, because α_4 integrins are also expressed on and used by eosinophils and monocytes and can contribute to neutrophil recruitment,[53–56] our data are only suggestive of a mechanism at this point and further testing is required.

As with all animal studies, the relation of the animal model to the human disease is not exactly known. Although several rodent models of inflammatory bowel disease are studied,[34–39] the SAMP-1/Yit model represents a novel, spontaneous model bearing many similarities with human Crohn's disease. The histological findings correspond to human Crohn's disease,[1,2,37] the disease is mainly localized to the terminal ileum, responds to conventional treatment like dexamethasone (present study), is dependent on the presence of gut flora,[37,41] and does not require targeted mutations or chemical interventions of questionable relevance to the (spontaneous) human disease. Therefore, we propose that our findings could be a foundation on which to build novel concepts of therapeutic interventions in Crohn's disease based on adhesion molecule blockade.

MAbs can have nonspecific effects, for example through Fc receptors.[57,58] We controlled for such effects by injecting separate groups of mice with isotype-matched control antibodies at the same dose. We did not observe any beneficial effects in isotype-treated animals compared with untreated mice. We also controlled for nonspecific effects of the adoptive transfer procedure by injecting a separate group of SCID mice with $CD4^+$ T cells from AKR donors, a control strain of mice with no propensity to develop a Crohn's-like disease. In the mice reconstituted with AKR $CD4^+$ T cells, no disease developed, suggesting that disease is specifically conferred by $CD4^+$ T cells from SAMP-1/Yit mice.

A technical limitation of this study is our inability to show that the dose of ICAM-1 and VCAM-1 antibodies injected is indeed saturating all binding sites. Based on experiments conducted with the dual radiolabeled antibody technique,[59] expressed ICAM-1 can be estimated to bind 24 μg antibody in a 30-g mouse, and VCAM-1 may bind 6 μg per 30-g mouse.[60] We have used this information to determine our injected dose and then added a safety margin. Our positive results suggest that our interventions were indeed effective. In the present study, we did not investigate the effect of adhesion molecule blockade on host defense. Previous studies in other models have suggested that host defense is maintained in rabbits treated with antibodies to adhesion molecules.[54] However, more studies of the combinations shown to be effective in this murine model of Crohn's disease are required. In this context, it is important to bear in mind that conventional treatment of the disease with immunosuppressants and corticosteroids also impairs host defense.

Interestingly, none of the 3-day treatment schemes resolved the chronic inflammation and villus remodeling as reflected by granuloma formation, muscular hypertrophy, and villus blunting and distortion. This could suggest that granulomata are relatively stable or villi might require a longer time than 3 days to heal. However, the complete resolution of chronic inflammation and villus blunting by dexamethasone treatment suggests otherwise. Apparently, adhesion molecule blockade of ICAM-1 and VCAM-1 or ICAM-1 and α_4 integrins eliminates active inflammation without impacting on secondary tissue changes, whereas dexamethasone leads to a more complete resolution of inflammation. Further studies will be necessary to address whether a synergistic effect may exist between blocking adhesion molecules and steroid treatment. A combination treatment might be able to save steroids and limit their side effects for the patient.

In conclusion, we show that a murine model of terminal ileitis that shares many characteristics with human Crohn's disease responds to treatment with antibodies to VCAM-1 and ICAM-1 or $\alpha 4$ integrins and ICAM-1 with significant improvement in active inflammation. This finding suggests that novel therapies for human Crohn's disease could be developed on the basis of these findings. Obviously, antibodies are an experimental tool and cannot always be used as clinical treatment modalities. However, the recent successes with antibodies to TNF-α[62] suggest that antibody treatment regimens can be successful. Both antibody-based[63] and nonantibody inhibitors of ICAM-1[64,65] have been developed. An antisense oligonucleotide ICAM-1 has been tested in human Crohn's disease and showed some benefit.[65] Development of such treatment schemes must balance the anti-inflammatory effect with a possible weakening of host defense. The present data suggest that adhesion molecule-based treatments can be successful.

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