Blocking Lymphocyte Localization to the Gastrointestinal Mucosa as a Therapeutic Strategy for Inflammatory Bowel Diseases


Eduardo J. Villablanca*


Barbara Cassani*


Ulrich H. von Andrian‡


J. Rodrigo Mora*

*Gastrointestinal Unit, Massachusetts General Hospital, Harvard Medical School, Boston; and ‡Immune Disease Institute, Children's Hospital Boston, and Department of Pathology, Harvard Medical School, Boston, Massachusetts

Lymphocyte migration (homing) to specific tissues has an important role during protective and pathological immune responses, including inflammatory bowel diseases. Lymphocytes use integrin $\alpha 4\beta 7$ and the chemokine receptor CCR9 to localize to the gastrointestinal mucosa; their respective ligands, mucosal addressin cell adhesion molecule-1 and CCL25, are displayed on endothelial cells in intestinal postcapillary venules. Although gastrointestinal-homing receptors are required for lymphocyte migration to the intestine in the noninflamed steady state, their role during inflammation is a matter of debate. Reagents designed to block interactions between these receptors and their ligands have had variable degrees of success in animal models of inflammatory bowel diseases and patients. We discuss the mechanisms involved in lymphocyte localization to the intestinal mucosa and how they can be applied to therapy for inflammatory bowel diseases.

Keywords: CCR9; $\alpha 4\beta 7$; IBD; Ulcerative Colitis; Crohn's Disease.

Lymphocytes localize to specific tissues during the protective immune response and in inflammatory disorders. Learning how these cells localize to different organs is important for understanding basic immunology as well as disease pathogenesis.

Circulating lymphocytes are exposed to extreme shear forces so they do not randomly adhere to endothelial cells[1]; instead, they express adhesion receptors for ligands expressed on endothelial cells. Adhesion usually takes place in postcapillary venules via a multistep process. First, lymphocytes are captured and loosely adhere to the endothelial cells (tethering and rolling, respectively), a step that usually requires selectins and their ligands, although the integrins $\alpha 4\beta 7$ and $\alpha 4\beta 1$ can also contribute to this step in some tissues. While lymphocytes are rolling they can be stimulated, generally via chemokine receptors (activation), which increases integrins' binding affinity and avidity. Integrin activation causes the lymphocytes to adhere to the endothelium (sticking) and then extravasation into noninflamed or inflamed tissues.

Lymphocyte migration and adhesion to specific tissues are determined by the combination of receptors involved in each step, rather than a single receptor and adhesive molecule. The diversity of receptors used in each step of the adhesion process allows for versatile and tissue-specific localization of lymphocytes, making lymphocyte adhesion amenable to modulation for therapeutic purposes.

The mechanisms that regulate lymphocyte homing to different tissues have been reviewed[2–4]; we focus on lymphocyte migration to the gastrointestinal (GI) mucosa and discuss how this process might be modulated in patients, to reduce GI inflammation.

Compartmentalized Homing to the Intestine

Naïve T and B cells constantly transit between the blood and secondary lymphoid organs, such as spleen, lymph nodes, and Peyer's patches. Upon activation in secondary lymphoid organs, naïve lymphocytes become effector and/or memory T and B cells and express receptors that control their migration to extralymphoid tissues, such as the skin, GI lamina propria, central nervous system (CNS), liver, and lungs.[5]

Abbreviations used in this paper: CD, Crohn's disease; CNS, central nervous system; GI, gastrointestinal; IBD, inflammatory bowel disease; ICAM-1, intercellular adhesion molecule-1; LFA, lymphocyte function antigen; MAdCAM1, mucosal addressin cell adhesion molecule-1; RA, retinoic acid; TNF-α, tumor necrosis factor–α; T_{REG}, regulatory T cells; UC, ulcerative colitis; VCAM-1, vascular cell adhesion molecule-1.

0016-5085/$36.00
doi:10.1053/j.gastro.2011.02.015

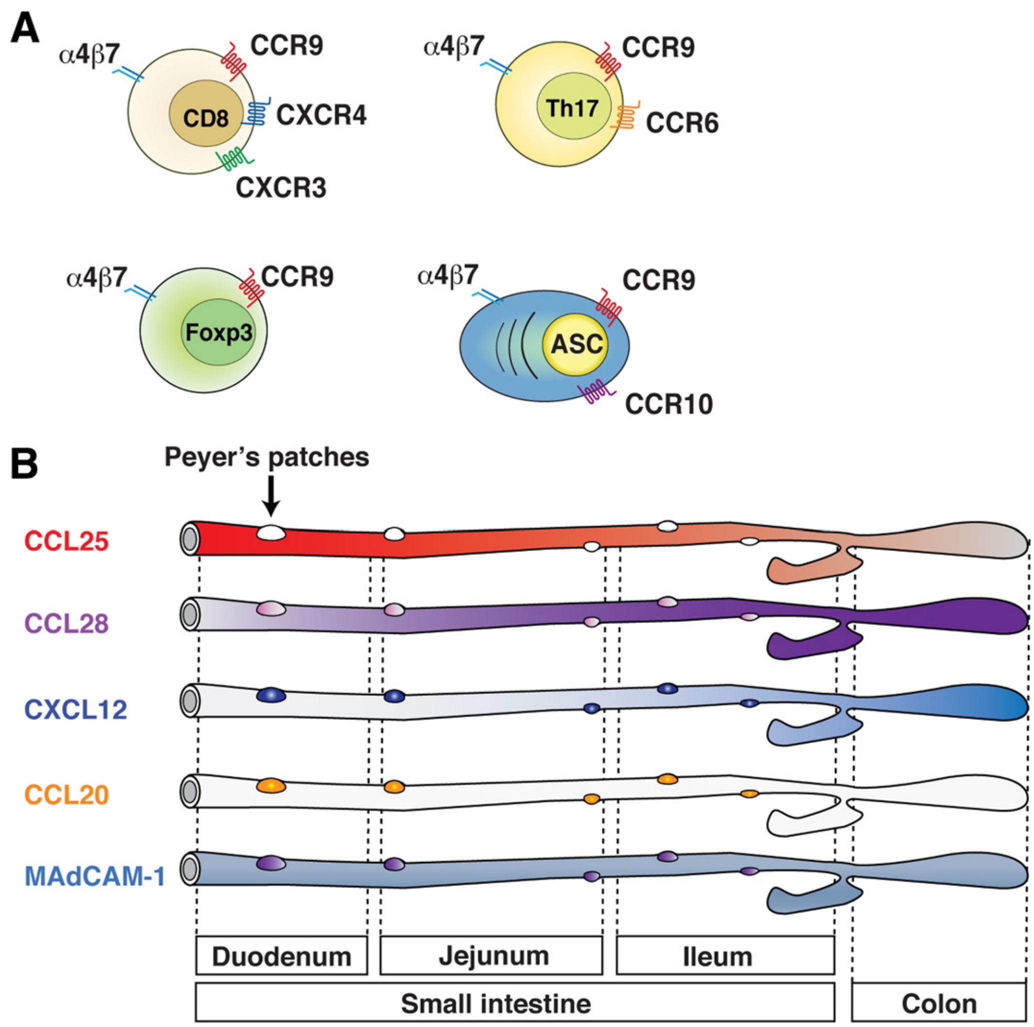

Figure 1. Different lymphocyte subsets use distinct homing receptors and ligands to localize to specific regions of the intestine. (*A*) Effector CD8$^+$ T cells use CCR9 and $\alpha 4\beta 7$, and possibly CXCR4 and/or CXCR3, to localize to the GI mucosa. Th17 cells might also use CCR6 to localize to small bowel, and IgA-secreting cells use CCR10 to localize to GI and other mucosal tissue compartments. (*B*) Expression of addressins varies throughout the intestine, even in the steady state. MAdCAM-1 is expressed along the whole intestine (small and large bowel), and it is up-regulated during inflammation. CCL25, a ligand for CCR9, is expressed in a proximal-to-distal gradient in the small bowel but absent from the colon. CCL28, a ligand for CCR10, is expressed mostly in colon and other mucosal sites; it regulates localization of IgA-secreting cells, but not T cells. CCL20, a ligand for CCR6, is most highly expressed in Peyer's patches and the small bowel, but also it is up-regulated in inflamed colon.

Although migration to secondary lymphoid organs occurs through the mechanism described here, lymphocyte migration to some extralymphoid tissues requires expression of specific receptors. T-cell localization in the GI mucosa and the skin—the largest surfaces in the body that are exposed to the external environment—has been well characterized. T-cell migration to the skin requires ligands for P- and E-selectins, CCR4, and the integrin lymphocyte function antigen (LFA)-1.[6]

In contrast to the skin, migration of T and B cells to the small intestine requires the integrin $\alpha 4\beta 7$ and CCR9, the induction of which depends on the vitamin A metabolite all-*trans* retinoic acid (RA)[3] (Figure 1). Localization to colon partially requires $\alpha 4\beta 7$, but not CCR9[7]; the chemokine receptor(s) required for lymphocyte migration to the colon have not been identified.

The ligand for CCR9, CCL25/TECK, is differentially distributed in a proximal-to-distal gradient in the small bowel; CD8$^+$ T cells localize to the ileum partially via CCR9-independent mechanisms (Figure 1).[7] Alternative candidates for T-cell migration to the small bowel include CXCR3 and CXCR4, the ligands of which (CXCL10 and CXCL12, respectively) are expressed in the GI mucosa.[8] Consistent with an in vivo role for these alternative chemokine pathways, *CXCR3*$^{-/-}$ mice have lower numbers of CD8$^+$ intestinal epithelial cells in the lamina propria[9]; blocking the interaction between CXCR4 and CXCL12 inhibits entry of T cells to the small intestine in steady-state and inflammatory conditions.[10]

Localization of lymphocytes to the colon differs in some ways from migration to the small bowel—it requires either $\alpha 4\beta 7$ or $\alpha 4\beta 1$, but not CCR9.[6,11] The ligand for $\alpha 4\beta 7$, mucosal addressin cell adhesion molecule-1 (MAdCAM-1), is expressed in small bowel and colon, whereas CCL25 is expressed in the small bowel only.[12,13] Moreover, although migration to the small intestine was impaired in *CCR9*$^{-/-}$ and β7 integrin chain-deficient ($\beta 7^{-/-}$) T-helper (Th)17, homing to the colon was reduced in only the $\beta 7^{-/-}$ Th17 cells.[14] Transfer of $\beta 7^{-/-}$ Th17 cells into severe combined immune-deficient mice induced less inflammation in the small and large bowel than transfer of wild-type Th17 cells (Table 1), whereas transfer of *CCR9*$^{-/-}$ Th17 induced less inflammation than wild-type cells in the small bowel only.[14] Together, these data indicate that CCR9 is required for T-cell migration and pathogenicity primarily in the small intestine, whereas $\alpha 4\beta 7$ is required for T-cell migration and pathogenicity in the small bowel and colon.

Migration of T cells to the intestinal mucosa also depends on their specific subset and phenotype. Recently activated $CD8^+$ T cells require CCR9 for migration to the small bowel, whereas effector $CD4^+$ T cells are less dependent on CCR9 for homing into this GI compartment.[15] Moreover, homing of *CCR6*$^{-/-}$ Th17 cells to Peyer's patches and small bowel was significantly reduced compared to wild-type Th17 cells, whereas homing of Th1 or $Foxp3^+$ regulatory T cells (T_{REG}) to these compartments did not require CCR6.[16] RA induces $\alpha 4\beta 7$ and CCR9 on T_{REG}[17,18]; however, mice given diets that did not contain vitamin A (and therefore lack RA synthesis) did not have decreased numbers of T_{REG} in the small bowel, although Th17 cells were markedly reduced in this compartment.[19] Moreover, T_{REG} isolated from mice depleted of dietary vitamin A were equally efficient in suppressing ileitis as T_{REG} from mice on a vitamin A–sufficient diet (or that received extra vitamin A).[20] However, these studies did not discriminate between thymus-derived or inducible T_{REG}. Further studies are needed to determine the in vivo roles of RA in localization of T_{REG} in the GI mucosa and their immunoregulatory functions there.

Another example of differential gut-homing requirements is cells that secrete IgA (IgA-secreting cells). CCR10 is expressed primarily by IgA-secreting cells, whereas cells that secrete antibodies against IgG or IgM do not express this receptor.[21] Moreover, IgA-secreting cells require CCR10 to localize to the intestine, although CCR10 is not expressed on gut-associated T cells.[4,21] In addition to CCR10, IgA-secreting cells also express CCR9; mice deficient in this receptor have reduced numbers of these cells in the small intestine.[22] Interestingly, blocking CCR9 prevented localization of IgA-secreting cells to the small bowel, whereas blocking either CCR10 or its mucosal ligand, CCL28, impaired their localization to small and large bowel.[23] Similar to T cells, RA was sufficient to induce CCR9 and $\alpha 4\beta 7$ (but not CCR10) on activated B cells and mice depleted of vitamin A had very low numbers of small bowel IgA-secreting cells.[19,24,25]

Aberrant Recruitment of Lymphocyte in IBDs

IBDs, which include Crohn's disease (CD) and ulcerative colitis (UC), are associated with a massive influx of immune cells into the GI tract. During disease development, altered production of proinflammatory cytokines induces expression of alternative adhesion receptors and chemokines on intestinal endothelial cells, which might allow lymphocytes to migrate to the intestine without expression of the receptors that normally regulate their localization in that compartment.[11] These alternative pathways of lymphocyte recruitment might have important implications for IBD therapy.

Studies of animal models and human tissues have indicated the role for gut-homing effector T cells in IBD pathogenesis. In experimental models of IBD, MAdCAM-1 is up-regulated in the intestinal lamina propria.[26–28,29] Similar MAdCAM-1 up-regulation is observed in active inflamed tissues from patients with CD or UC, which is associated with increased numbers of $\alpha 4\beta 7^+$ T cells compared with normal tissues.[30,31] Deficiency of β7 integrin subunit inhibits inflammation in a mouse model of CD[32] and antibodies against β7 or MAdCAM-1 reduced inflammation in mice with trinitrobenzene sulfonic acid−induced or cell transfer-induced colitis[33,34] (Table 1). Blocking α4 or $\alpha 4\beta 7$ reduced colitis in a nonhuman primate model of IBD.[35,36] Additional mechanistic insights have come from studies of SAMP1/Yit mice, which spontaneously develop CD-like ileitis. Although SAMP1/Yit mice deficient in β7 have reduced intestinal inflammation,[37] antibodies that block $\alpha 4\beta 7$ or MAdCAM-1 did not decrease the inflammation; only combined blockade of vascular cell adhesion molecule 1 (VCAM-1) and MAdCAM-1 significantly improved ileitis.[29]

Blocking $\alpha 4\beta 1$, a collagen-binding integrin that is up-regulated in inflamed tissues, reduced trinitrobenzene sulfonic acid–induced colitis in mice.[38] Mice with dextran sodium sulfate−induced colitis have varied results–some studies reported that blockers of MAdCAM-1 reduced inflammation,[33,39,40] whereas others showed that development of colitis required $\alpha 4\beta 7$–VCAM-1 and LFA-1–intercellular adhesion molecule 1 (ICAM-1) interactions, but not $\alpha 4\beta 7$–MAdCAM-1.[41,42] Pathogenic effector T cells might not require only interaction between lymphocyte $\alpha 4\beta 7$ and endothelial cell MAdCAM-1 to promote chronic inflammation, but other integrins that mediate immune cell localization during general inflammation might fulfill redundant roles in intestinal pathology.

SAMP1/Yit mice have increased numbers of IgA-secreting cells in the mesenteric lymph nodes and lamina propria.[43] Adoptive transfer of B cells and T cells from SAMP1/Yit into severe combined immune-deficient mice increased ileitis, compared with transfer of only T cells.[43] Moreover, B cells required $\alpha 4\beta 7$ to exacerbate ileitis,[37] indicating that B-cell localization to the GI tract might also be involved in IBD pathogenesis.

Table 1. Role of Gut-Homing Receptors in Experimental Inflammatory Bowel Disease Models

					Blocking homing receptors		
Model	Gut segment	Pathogenic cells	Advantages	Limitations	$\alpha 4\beta 7$/MAdCAM-1	CCR9/CCL25	References
Mouse DSS colitis	Colon	Innate immunity T/B-cell–independent (occurs in RAG1$^{-/-}$ mice)	Easy to set up, fast readout	Mostly colon inflammation, acute disease (can also be made chronic), T/B-cell–independent, little resemblance to human IBD	Variable, with only some studies showing an effect in decreasing inflammation	ND	33, 39–42
Mouse TNBS colitis	Colon	Th1	Easy to set up, fast readout	Mostly colon, acute disease, little resemblance to human IBD	Decreases colitis	ND	33
Naïve CD4 T-cell transfer into RAG1$^{-/-}$ or SCID mice	Colon	Th1, Th17?	Chronic disease, easy to set up, reproducibility	Mostly colon inflammation	Decreases colitis	ND	34
Gut-tropic Th17 cell transfer into RAG1$^{-/-}$ mice	Ileum and colon	Ex vivo differentiated gut-homing Th17	Involvement of small bowel (ileitis) and colon	Need transfer of ex vivo differentiated gut-homing Th17 cells	Decreases ileitis and colitis	Only decreases ileitis	14
Cotton-top tamarin	Colon	Th1?	Nonhuman primate	Cost, logistical limitations	Decreases colitis	ND	35, 36
TNFARE mice (TNF-α overproduction)	Ileum	Th1	Affects small bowel (ileitis), chronic disease, model for human CD	Logistical (mice are noncommercially available)	Suppresses ileitis	No effect	32
Samp1/Yit mice	Ileum	Th2, Th17, B cells	Affects small bowel (ileitis), chronic disease, model for human CD		Reduced ileitis in SAMP1/Yit b7$^{-/-}$ mice Decreased ileitis when blocking both MAdCAM-1 and VCAM-1	Can prevent inflammation, but only early in disease	29, 37, 50

DSS, dextran sodium sulfate; ND, not determined; RAG, recombination activating gene; SCID, severe combined immune-deficient; Th, T-helper; TNBS, trinitrobenzene sulfonic acid; VCAM-1, vascular cell adhesion molecule 1.

T_{REG} cells are believed to prevent or even cure intestinal inflammation, based on studies from different models of IBD. However, the precise role of $\alpha 4\beta 7$ and CCR9 in trafficking and function of T_{REG} cells during GI inflammation is unclear. T_{REG} cells seem to require $\beta 7$-independent pathways—rather, those that involve CCR7 and CCR4—for their immune suppressive functions and to prevent colitis.[44–46] These alternative migratory pathways might allow T_{REG} cell function in lymphoid compartments, such as prophylactic suppression before the onset of inflammation in MLN or Peyer's patches. However, T_{REG} cells might need GI homing receptors to suppress immune activity in the lamina propria during active inflammation,[47] which is probably most relevant for development of therapeutics.

CCL5 and CCR5 are also up-regulated in experimental models of ileitis and mediate the specific recruitment of T_{REG} cells and some subsets of effector $CD4^+$ T cells.[48] These alternative chemokine pathways could account for the observation that blocking CCL25 or CCR9 is only effective at early stages of disease, even though expression of CCL25 increases in the small bowel of patients with CD.[49] Other, perhaps non–GI-specific, chemokine signals might mediate lymphocyte homing at later stages during inflammation.[50] Human lamina propria and intraepithelial lymphocytes also express CXCR3, CX3CR1, and CCR2, and levels of their ligands are increased in tissues of patients with CD.[51] Moreover, recruitment of T cells, monocytes, and DC to the inflamed mucosa might involve CX3CL1 and its receptor CX3CR1, which contributes to pathogenesis of IBD.[52–54]

Some of the extraintestinal pathologies associated with IBD might arise from aberrant homing of immune cells. For instance, MAdCAM-1 and CCL25 are up-regulated in the liver during primary sclerosing cholangitis, a chronic disease characterized by progressive inflammation and scarring of the bile ducts. Primary sclerosing cholangitis has been associated with UC in epidemiologic studies.[55]

Therapies for IBD

Patients with IBD usually require life-long therapy with corticosteroids and other immunosuppressive drugs. Choice of therapy depends on the primary clinical goal (induction or maintenance of remission), the extent and severity of disease, the response to current or prior treatments, and the occurrence of side effects (summarized in Supplementary Tables 1-2). Many drugs for IBD can have serious adverse effects, and some patients become refractory to treatment during disease progression and require surgery. Therefore, new therapeutic approaches, that target specific inflammatory mediators, are needed.

Although the primary causes of IBD are not clear, many molecules that are involved in disease pathogenesis have been identified as targets for therapy. Therapeutics that have been developed include inhibitors of T-cell activation, costimulatory pathways, proinflammatory cytokine receptors, Th1 polarization, cytokines and their regulatory proteins, growth hormone, and growth factors (Supplementary Tables 3-4).

Although many of these biologic agents showed efficacy in preclinical studies, most of them have not shown efficacy in clinical trials, or have caused significant side effects.[56] Antibodies to the cytokine tumor necrosis factor–α (TNF-α) have been the most effective, and are currently used to treat patients with refractory moderate-to-severe active CD or UC. Infliximab binds the soluble bioactive and membrane-bound forms of human TNF-α and reduces its toxicity. Although infliximab is effective in reducing the symptoms of IBDs, its immunosuppressive effects predispose patients to infections and increase risk of malignancies, such as lymphomas.[57,58]

Blocking Adhesion Receptors

Molecules that mediate lymphocyte localization to the GI mucosa, in the steady state or during development of inflammatory diseases such as IBD, are attractive targets for drug development. Antibodies or compounds that selectively block homing receptors,[11] or reagents that sequester lymphocytes in secondary lymphoid organs (to prevent their migration to sites of inflammation),[59] have shown efficacy in animal models and in clinical trials for psoriasis,[60,61] asthma,[62] graft-vs-host disease,[63] and multiple sclerosis.[59]

Because $\alpha 4\beta 7$ and CCR9 are the primary mediators of lymphocyte migration to the intestine, reagents that block their function should reduce inflammation in the intestinal mucosa, yet cause low levels of systemic immunosuppression. Agents developed for treatment of IBD disrupt interactions between LFA-1 and ICAM-1, $\alpha 4\beta 1$, and VCAM-1, as well as $\alpha 4\beta 7$ and MAdCAM-1 (Figure 2, Table 2).

The first successful clinical use of anti-ICAM-1 was in treatment of rheumatoid arthritis.[64] Antibodies against ICAM-1 or antisense oligonucleotides that disrupt expression of ICAM-1 showed efficacy in mouse models of IBD, including dextran sodium sulfate–induced colitis and SAMP-1/Yit mice ileitis.[65,66] Interestingly, in SAMP-1/Yit mice, anti-ICAM-1 was only effective when administered in combination with anti–VCAM-1 or anti-$\alpha 4$ integrins, indicating redundancy between LFA-1/ICAM-1 and $\alpha 4\beta 1$/VCAM-1 pathways during inflammation in mice. Clinical trials that investigated the effects of reagents against ICAM-1 in patients with IBD included investigation of alicaforsen (ISIS 2302), an antisense oligonucleotide that prevents expression of ICAM-1. Whereas an early-stage clinical trial suggested a therapeutic potential for alicaforsen in patients with mild-to-moderate active CD,[67] two subsequent, larger, multicenter trials failed to demonstrate significant efficacy.[68,69] Despite this setback, patients treated with alicaforsen, in an enema formulation, had significant improvements in distal UC in a randomized, placebo-controlled trial.[70] However, given the important role of LFA-1 interaction

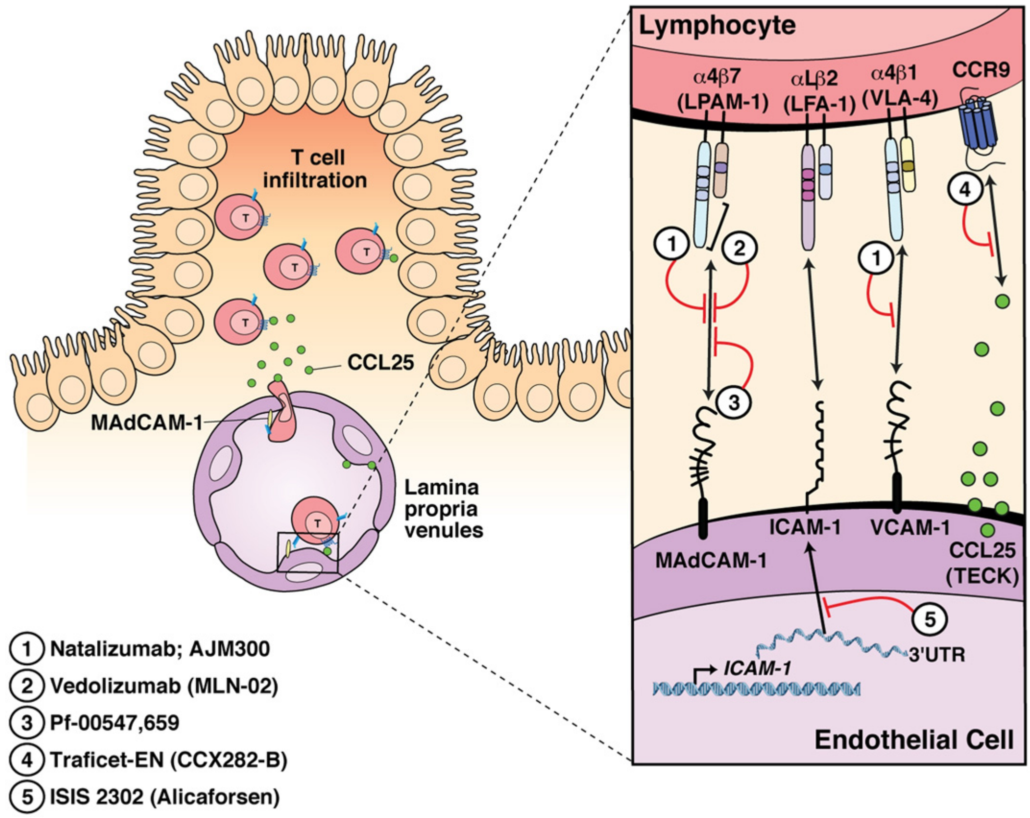

Figure 2. Interfering with homing receptors as therapy for inflammatory bowel diseases. Natalizumab is a monoclonal antibody (mAb) that blocks the integrins $\alpha4\beta7$ and $\alpha4\beta1$, preventing their binding to MAdCAM-1 and VCAM-1, respectively. Similarly, AJM300 is an orally bioavailable antagonist of the integrin $\alpha4$ subunit. The mAbs vedolizumab and Pf-00547,659 bind specifically to $\alpha4\beta7$ and MAdCAM-1, respectively, blocking their interaction. Traficet-EN (CCX282-B) is an orally bioavailable selective antagonist of CCR9 that blocks its functional interaction with CCL25. Alicaforsen (ISIS 2302) is an antisense oligodeoxynucleotide that binds to the 3′ UTR portion of the *ICAM1* messenger RNA and prevents its translation.

with ICAM-1 in leukocyte localization to many lymphoid and nonlymphoid tissues, as well as in T-cell activation,[11] it is likely that blockers of ICAM-1 will cause significant systemic immunosuppression.

Natalizumab is a recombinant, humanized, monoclonal IgG4 against the alpha-4 integrin chain; it inhibits MAdCAM-1 binding to integrin $\alpha4\beta7$ and VCAM-1 binding to integrin $\alpha4\beta1$.[71] In placebo-controlled, randomized trials, 40% patients with moderate-to-severe CD responded to natalizumab and went to remission, compared to 8% in the group that received placebo.[72] However, phase III clinical trials that included clinical response, remission, and maintenance as end points showed that the drug was more effective when given in combination with other immunosuppressants or before therapy with an anti–TNF-α reagent.[73,74] Because blockers of the alpha-4 integrin chain probably do not affect T cells that have already localized to intestinal tissues, natalizumab might not be sufficient to effectively reduce ongoing inflammation—its combination with other immunosuppressant drugs might be required. natalizumab has also shown potential for treatment of UC,[75] probably due to its ability to block $\alpha4\beta7$ and $\alpha4\beta1$ (integrins involved in localization of lymphocytes to the colon).

Natalizumab has also been used to treat patients with multiple sclerosis,[76] based on the role of $\alpha4\beta1$ interaction with VCAM-1 in leukocyte homing to the CNS and experimental allergic encephalomyelitis.[77] However, cases of progressive multifocal leukoencephalopathy—a rare and often fatal opportunistic infection of the CNS— have developed in some patients given natalizumab (approximate incidence 1/1000), raising concerns about its safety.[78,79] Sporadic cases of melanoma have also been reported in patients treated with natalizumab, which might be associated with impaired immunosurveillance of the skin following $\alpha4\beta1$ blockade.[80,81] However, larger cohorts of patients that have received natalizumab need to be studied to determine more precisely the incidence of these rare side effects. Similar safety concerns might apply to an orally bioavailable inhibitor of the alpha-4 integrin chain (AJM300), which has also shown to be effective in patients with active CD.[82] Some cases of progressive multifocal leukoencephalopathy also occurred in patients with psoriasis who were treated with efalizumab, a monoclonal antibody against LFA-1.[83] Reagents that selectively block homing of lymphocytes to the GI without affecting immunosurveillance in other tissues (including the CNS) are urgently required for IBD.

Table 2. Targeting Homing Receptors in Inflammatory Bowel Disease

Target	Name	Indication	Clinical phase	Mechanism	Advantages	Side effects	References
Adhesion molecules							
Human ICAM-1 antisense oligodeoxynucleotide	Alicaforsen (ISIS 2302)	CD, UC	Phase II/III	Reduction of ICAM-1 protein expression	Clinical improvements and well-tolerated	Injection/infusion site reactions; systemic immunosuppression	64–66, 69, 70
Humanized IgG4 mAb anti-alpha-4 integrin	Natalizumab	CD, UC	Phase IV FDA-approved	Inhibition of $\alpha4\beta7$/MAdCAM-1 interaction and $\alpha4\beta1$/VCAM-1 binding	Long-term clinical response and/or remission following the withdrawal of concomitant corticosteroids; well-tolerated	Rare cases of progressive multifocal leukoencephalopathy; rare cases of melanoma?	71–79
Orally bioavailable alpha-4 integrin inhibitor	AJM300	CD, UC?	Phase II	Inhibition of $\alpha4\beta7$/MAdCAM-1 interaction and $\alpha4\beta1$/VCAM-1 binding	Orally bioavailable	Same as natalizumab?	82
Humanized IgG1 mAb anti-$\alpha4\beta7$ integrin	Vedolizumab (MLN-02)	CD, UC	Phase II	Inhibition of MAdCAM-1–mediated leukocyte adhesion	Good clinical response and well-tolerated, lack of effects on CNS and skin homing/immunosurveillance	Infusion reaction with angioedema, nausea, and nasopharyngitis; efficacy limited by development of anti-drug antibody	84, 85
Human IgG2 mAb anti-MAdCAM	PF-00547,659	UC, CD?	Phase I/II	Inhibition of $\alpha4\beta7$/MAdCAM-1 interaction	Clinical improvements and well-tolerated	No major side effects reported	86
Chemokine receptors							
Orally bioavailable antagonist for CCR9	Traficet-EN (CCX282-B)	CD, GVHD? Celiac disease? Intestinal transplant?	Phase III	Inhibition of CCL25/CCR9 functional interaction	Clinical remission; well-tolerated; orally bioactive; no risk of skin delayed-type hypersensitivity reactions; no increased risk of systemic infection; no formation of anti-human Ab	No major side effects reported	50, 87, 88

Ab, antibody; FDA, Food and Drug Administration; GVHD, graft-vs-host disease; mAb, monoclonal antibody.

Vedolizumab (MLN-02) is a humanized monoclonal IgG1 against integrin $\alpha4\beta7$. In a phase II trial in 181 patients with UC, remission rates were significantly higher among subjects treated with vedolizumab than those given placebo.[84] Another placebo-controlled trial of 185 patients with mild-to-moderate active CD showed that vedolizumab was significantly more effective than placebo at inducing remission in patients with CD.[85]

A monoclonal antibody against MAdCAM-1 (Pf-0054,659, human IgG2) is being tested in a phase I/II clinical trial of patients with UC. Although the study includes a small number of patients, endoscopic examinations identified improvements among patients treated with anti-MAdCAM-1, without major side effects.[86]

Nevertheless, because $\alpha4\beta1$ might also have a role in chronic intestinal inflammation,[29,41,42] it is possible that selective targeting of $\alpha4\beta7$ or MAdCAM-1 might not be as effective as reagents designed to block the alpha-4 integrin chain.

Chemokines as Therapeutic Targets

Blocking either CCL25 or CCR9 during early stages of ileitis in SAMP1/Yit mice reduced inflammation, whereas no effect was observed when mice were given the reagents at later stages of disease progression.[50] Moreover, administration of CCX282 (Traficet-EN), an orally bioavailable antagonist of CCR9, reduced the inflammatory response in mice when given before or after gut inflammation induced by TNF-α overexpression.[87]

CCX282 is being tested in trials of patients with CD and refractory celiac disease (Figure 2, Table 2); preliminary efficacy and safety evaluations look promising.[88] In a phase II trial, 74 patients with CD were given either CCX282 or placebo for 28 days. Fifty-eight percent of patients had a significant reduction in CD scores, compared with 31% in the placebo group; in the CCX282 group, the therapeutic effect was associated with reduced levels of proinflammatory cytokines and C-reactive protein.[88] A subsequent phase II/III trial showed significant improvement in the CCX282 group; disease scores were reduced in 81% of patients and 41% experienced clinical remission—effects that were maintained even upon withdrawal of corticosteroids.[88]

Results from larger phase III trials of CCX282 for CD and UC are pending. Although the drug was generally well-tolerated and not associated with an increased risk of infection, a large cohort of patients must be followed for a long time period to exclude risk for rare diseases such as progressive multifocal leukoencephalopathy. The fact that CCX282 is orally bioavailable offers a clear advantage to parenteral therapies, decreasing the cost of the treatment, eliminating morbidity associated with parenteral administration, and potentially increasing compliance.

Interestingly, tolerogenic plasmacytoid DC also express CCR9,[89] which seems to be required for localization of these cells to the small bowel.[90] Inhibitors of CCR9 might affect migration of plasmacytoid DC and their tolerogenic functions in the intestine, with potential effects that should be considered and explored in models of IBD pathogenesis.

Conclusions

Although the exact cellular and molecular mechanisms of IBD pathogenesis are undefined, lymphocyte homing has an important role. Improved understanding of lymphocyte localization to the noninflamed and inflamed intestinal mucosa has led to specific and effective therapies for IBD and improved the benefit-to-risk profile for patients.

Nevertheless, results from studies of animal models of IBD have identified alternative homing receptors that, in addition to $\alpha4\beta7$ and/or CCR9, have roles in lymphocyte migration to the GI tract and might contribute to inflammation; these redundant homing pathways could account for the varying degrees of effectiveness of reagents that target GI-specific homing receptors in clinical trials. It is important to better define which receptors, adhesion molecules, and chemokine pathways contribute to chronic intestinal inflammation in humans. It is also important to determine the precise role of T_{REG} cells in intestinal inflammation and whether GI-specific reagents that interfere with lymphocyte adhesion affect T_{REG} functions. Combination therapies, which target more than one step in adhesion of lymphocytes to the intestinal epithelium, might be the most effective strategy for IBD. Combinations such as anti–$\alpha4\beta7$ and antagonists of CCR9 could have additive effects to reduce inflammation in patients with IBD.

Supplementary Material

Note: The first 50 references associated with this article are available below in print. The remaining references accompanying this article are available online only with the electronic version of the article. Visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi:10.1053/j.gastro.2011.02.015.

References

1. von Andrian UH, Mackay CR. T-cell function and migration. Two sides of the same coin. N Engl J Med 2000;343:1020–1034.
2. Mora JR, von Andrian UH. Differentiation and homing of IgA-secreting cells. Mucosal Immunol 2008;1:96–109.
3. Mora JR, Iwata M, von Andrian UH. Vitamin effects on the immune system: vitamins A and D take centre stage. Nat Rev Immunol 2008;8:685–698.
4. Mora JR. Homing imprinting and immunomodulation in the gut: role of dendritic cells and retinoids. Inflamm Bowel Dis 2008;14: 275–289.
5. Masopust D, Vezys V, Marzo AL, et al. Preferential localization of effector memory cells in nonlymphoid tissue. Science 2001;291: 2413–2417.
6. Mora JR, von Andrian UH. T-cell homing specificity and plasticity: new concepts and future challenges. Trends Immunol 2006;27: 235–243.
7. Stenstad H, Svensson M, Cucak H, et al. Differential homing mechanisms regulate regionalized effector CD8alphabeta+ T cell accumulation within the small intestine. Proc Natl Acad Sci U S A 2007;104:10122–10127.
8. Wright N, Hidalgo A, Rodriguez-Frade JM, et al. The chemokine stromal cell-derived factor-1 alpha modulates alpha 4 beta 7 integrin-mediated lymphocyte adhesion to mucosal addressin cell adhesion molecule-1 and fibronectin. J. Immunol 2002;168: 5268–5277.
9. Annunziato F, Cosmi L, Liotta F, et al. CXCR3 and alphaEbeta7 integrin identify a subset of CD8+ mature thymocytes that share phenotypic and functional properties with CD8+ gut intraepithelial lymphocytes. Gut 2006;55:961–968.
10. Oyama T, Miura S, Watanabe C, et al. CXCL12 and CCL20 play a significant role in mucosal T-lymphocyte adherence to intestinal microvessels in mice. Microcirculation 2007;14:753–766.
11. Rodrigo Mora J, Von Andrian UH. Specificity and plasticity of memory lymphocyte migration. Curr Top Microbiol Immunol 2006;308:83–116.
12. Kunkel EJ, Campbell JJ, Haraldsen G, et al. Lymphocyte CC chemokine receptor 9 and epithelial thymus-expressed chemokine (TECK) expression distinguish the small intestinal immune compartment: epithelial expression of tissue-specific chemokines as an organizing principle in regional immunity. J Exp Med 2000;192:761–768.
13. Papadakis KA, Prehn J, Nelson V, et al. The role of thymus-expressed chemokine and its receptor CCR9 on lymphocytes in the regional specialization of the mucosal immune system. J Immunol 2000;165:5069–5076.
14. Wang C, Kang SG, HogenEsch H, et al. Retinoic acid determines the precise tissue tropism of inflammatory Th17 cells in the intestine. J Immunol 2010;184:5519–5526.
15. Stenstad H, Ericsson A, Johansson-Lindbom B, et al. Gut associated lymphoid tissue primed CD4+ T cells display CCR9 dependent and independent homing to the small intestine. Blood 2006;107:3447–3454.
16. Wang C, Kang SG, Lee J, et al. The roles of CCR6 in migration of Th17 cells and regulation of effector T-cell balance in the gut. Mucosal Immunol 2009;2:173–183.
17. Kang SG, Lim HW, Andrisani OM, et al. Vitamin A metabolites induce gut-homing FoxP3+ regulatory T cells. J Immunol 2007; 179:3724–3733.
18. Benson MJ, Pino-Lagos K, Rosemblatt M, et al. All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. J Exp Med 2007:1775–1774.
19. Cha HR, Chang SY, Chang JH, et al. Downregulation of Th17 cells in the small intestine by disruption of gut flora in the absence of retinoic acid. J Immunol 2010;184:6799–6806.
20. Kang SG, Wang C, Matsumoto S, et al. High and low vitamin A therapies induce distinct FoxP3+ T-cell subsets and effectively control intestinal inflammation. Gastroenterology 2009;137: 1391–1402 e1-6.
21. Kunkel EJ, Butcher EC. Plasma-cell homing. Nat Rev Immunol 2003;3:822–829.
22. Pabst O, Ohl L, Wendland M, et al. Chemokine receptor CCR9 contributes to the localization of plasma cells to the small intestine. J Exp Med 2004;199:411–416.
23. Hieshima K, Kawasaki Y, Hanamoto H, et al. CC chemokine ligands 25 and 28 play essential roles in intestinal extravasation of IgA antibody-secreting cells. J Immunol 2004;173:3668–3675.
24. Mora JR, Iwata M, Eksteen B, et al. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. Science 2006; 314:1157–1160.

25. Uematsu S, Fujimoto K, Jang MH, et al. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. Nat Immunol 2008;9:769–776.
26. Shigematsu T, Specian RD, Wolf RE, et al. MAdCAM mediates lymphocyte-endothelial cell adhesion in a murine model of chronic colitis. Am J Physiol Gastrointest Liver Physiol 2001;281: G1309–G1315.
27. Kawachi S, Morise Z, Jennings SR, et al. Cytokine and adhesion molecule expression in SCID mice reconstituted with CD4+ T cells. Inflamm Bowel Dis 2000;6:171–180.
28. Matsuzaki K, Tsuzuki Y, Matsunaga H, et al. In vivo demonstration of T lymphocyte migration and amelioration of ileitis in intestinal mucosa of SAMP1/Yit mice by the inhibition of MAdCAM-1. Clin Exp Immunol 2005;140:22–31.
29. Rivera-Nieves J, Olson T, Bamias G, et al. L-selectin, alpha4beta1, and alpha4beta7 integrins participate in CD4+ T cell recruitment to chronically inflamed small intestine. J Immunol 2005;174:2343–2352.
30. Souza HS, Elia CC, Spencer J, et al. Expression of lymphocyte-endothelial receptor-ligand pairs, alpha4beta7/MAdCAM-1 and OX40/OX40 ligand in the colon and jejunum of patients with inflammatory bowel disease. Gut 1999;45:856–863.
31. Arihiro S, Ohtani H, Suzuki M, et al. Differential expression of mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in ulcerative colitis and Crohn's disease. Pathol Int 2002;52:367–374.
32. Apostolaki M, Manoloukos M, Roulis M, et al. Role of beta7 integrin and the chemokine/chemokine receptor pair CCL25/CCR9 in modeled TNF-dependent Crohn's disease. Gastroenterology 2008;134:2025–2035.
33. Goto A, Arimura Y, Shinomura Y, et al. Antisense therapy of MAdCAM-1 for trinitrobenzenesulfonic acid-induced murine colitis. Inflamm Bowel Dis 2006;12:758–765.
34. Picarella D, Hurlbut P, Rottman J, et al. Monoclonal antibodies specific for beta 7 integrin and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) reduce inflammation in the colon of scid mice reconstituted with CD45RBhigh CD4+ T cells. J Immunol 1997;158:2099–2106.
35. Hesterberg PE, Winsor-Hines D, Briskin MJ, et al. Rapid resolution of chronic colitis in the cotton-top tamarin with an antibody to a gut-homing integrin alpha 4 beta 7. Gastroenterology 1996; 111:1373–1380.
36. Podolsky DK, Lobb R, King N, et al. Attenuation of colitis in the cotton top tamarin by anti-a4 integrin monoclonal antibody. J Clin Invest 1993;92:372–380.
37. Gorfu G, Rivera-Nieves J, Hoang S, et al. Beta7 integrin deficiency suppresses B cell homing and attenuates chronic ileitis in SAMP1/YitFc mice. J Immunol 2010;185:5561–5568.
38. Fiorucci S, Mencarelli A, Palazzetti B, et al. Importance of innate immunity and collagen binding integrin alpha1beta1 in TNBS-induced colitis. Immunity 2002;17:769–780.
39. Farkas S, Hornung M, Sattler C, et al. Blocking MAdCAM-1 in vivo reduces leukocyte extravasation and reverses chronic inflammation in experimental colitis. Int J Colorectal Dis 2006;21:71–78.
40. Kato S, Hokari R, Matsuzaki K, et al. Amelioration of murine experimental colitis by inhibition of mucosal addressin cell adhesion molecule-1. J Pharmacol Exp Ther 2000;295:183–189.
41. Taniguchi T, Tsukada H, Nakamura H, et al. Effects of the anti-ICAM-1 monoclonal antibody on dextran sodium sulphate-induced colitis in rats. J Gastroenterol Hepatol 1998;13:945–949.
42. Soriano A, Salas A, Sans M, et al. VCAM-1, but not ICAM-1 or MAdCAM-1, immunoblockade ameliorates DSS-induced colitis in mice. Lab Invest 2000;80:1541–1551.
43. Olson TS, Bamias G, Naganuma M, et al. Expanded B cell population blocks regulatory T cells and exacerbates ileitis in a murine model of Crohn disease. J Clin Invest 2004;114:389–398.
44. Denning TL, Kim G, Kronenberg M. Cutting edge: CD4+CD25+ regulatory T cells impaired for intestinal homing can prevent colitis. J Immunol 2005;174:7487–7491.
45. Schneider MA, Meingassner JG, Lipp M, et al. CCR7 is required for the in vivo function of CD4+ CD25+ regulatory T cells. J Exp Med 2007;204:735–745.
46. Yuan Q, Bromley SK, Means TK, et al. CCR4-dependent regulatory T cell function in inflammatory bowel disease. J Exp Med 2007;204:1327–1334.
47. Mottet C, Uhlig HH, Powrie F. Cutting edge: cure of colitis by CD4+CD25+ regulatory T cells. J Immunol 2003;170:3939–3943.
48. Kang SG, Piniecki RJ, Hogenesch H, et al. Identification of a chemokine network that recruits FoxP3(+) regulatory T cells into chronically inflamed intestine. Gastroenterology 2007;132:966–981.
49. Papadakis KA, Prehn J, Moreno ST, et al. CCR9-positive lymphocytes and thymus-expressed chemokine distinguish small bowel from colonic Crohn's disease. Gastroenterology 2001;121:246–254.
50. Rivera-Nieves J, Ho J, Bamias G, et al. Antibody blockade of CCL25/CCR9 ameliorates early but not late chronic murine ileitis. Gastroenterology 2006;131:1518–1529.

Received November 5, 2010. Accepted February 7, 2011.

Reprint requests

Address requests for reprints to: Ulrich H. von Andrian, MD, PhD, Immune Disease Institute and Children's Hospital, Harvard Medical School, Boston, Massachusetts. e-mail: uva@hms.harvard.edu; fax: (617) 432-6829 or J. Rodrigo Mora, MD, PhD, Gastrointestinal Unit, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts. e-mail: j_rodrigo_mora@hms.harvard.edu; fax: (617) 849-5771.

Acknowledgments

We are grateful to Allison McNulty for editorial assistance. JRM is indebted to Ingrid Ramos for constant support.

Conflicts of interest

The authors disclose no conflicts.

Funding

EJV was supported by a postdoctoral fellowship from Crohn's & Colitis Foundation of America (CCFA). BC was supported by an EMBO postdoctoral fellowship. UHvA was supported by grants from National Institutes of Health (NIH). JRM was supported by grants from CCFA, Cancer Research Institute (CRI), Howard H. Goodman (MGH), Massachusetts Life Science Center (MLSC), and NIH DP2 2009A054301.

References (Online Only)

51. Kunkel EJ, Butcher EC. Chemokines and the tissue-specific migration of lymphocytes. Immunity 2002;16:1–4.
52. Sans M, Danese S, de la Motte C, et al. Enhanced recruitment of CX3CR1+ T cells by mucosal endothelial cell-derived fractalkine in inflammatory bowel disease. Gastroenterology 2007;132:139–153.
53. Fong AM, Robinson LA, Steeber DA, et al. Fractalkine and CX_3CR1 mediate a novel mechanism of leukocyte capture, firm adhesion, and activation under physiologic flow. J Exp Med 1998;188:1413–1419.
54. Kostadinova FI, Baba T, Ishida Y, et al. Crucial involvement of the CX3CR1-CX3CL1 axis in dextran sulfate sodium-mediated acute colitis in mice. J Leukoc Biol 2010;88:133–143.
55. Adams DH, Eksteen B. Aberrant homing of mucosal T cells and extra-intestinal manifestations of inflammatory bowel disease. Nat Rev Immunol 2006;6:244–251.
56. Stallmach A, Hagel S, Bruns T. Adverse effects of biologics used for treating IBD. Best Pract Res Clin Gastroenterol 2010;24:167–182.
57. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med 2010;362:1383–1395.
58. Diak P, Siegel J, La Grenade L, et al. Tumor necrosis factor alpha blockers and malignancy in children: forty-eight cases reported to the Food and Drug Administration. Arthritis Rheum 2010;62:2517–2524.
59. Massberg S, von Andrian UH. Fingolimod and sphingosine-1-phosphate—modifiers of lymphocyte migration. N Engl J Med 2006;355:1088–1091.
60. Schon MP, Drewniok C, Boehncke WH. Targeting selectin functions in the therapy of psoriasis. Curr Drug Targets Inflamm Allergy 2004;3:163–168.
61. Lebwohl M, Tyring SK, Hamilton TK, et al. A novel targeted T-cell modulator, efalizumab, for plaque psoriasis. N Engl J Med 2003;349:2004–2013.
62. Sawicka E, Zuany-Amorim C, Manlius C, et al. Inhibition of Th1- and th2-mediated airway inflammation by the sphingosine 1-phosphate receptor agonist FTY720. J Immunol 2003;171:6206–6214.
63. Waldman E, Lu SX, Hubbard VM, et al. Absence of beta7 integrin results in less graft-versus-host disease because of decreased homing of alloreactive T cells to intestine. Blood 2006;107:1703–1711.
64. Kavanaugh AF, Davis LS, Nichols LA, et al. Treatment of refractory rheumatoid arthritis with a monoclonal antibody to intercellular adhesion molecule 1. Arthritis Rheum 1994;37:992–999.
65. Hamamoto N, Maemura K, Hirata I, et al. Inhibition of dextran sulphate sodium (DSS)-induced colitis in mice by intracolonically administered antibodies against adhesion molecules (endothelial leucocyte adhesion molecule-1 (ELAM-1) or intercellular adhesion molecule-1 (ICAM-1)). Clin Exp Immunol 1999;117:462–468.
66. Burns RC, Rivera-Nieves J, Moskaluk CA, et al. Antibody blockade of ICAM-1 and VCAM-1 ameliorates inflammation in the SAMP-1/Yit adoptive transfer model of Crohn's disease in mice. Gastroenterology 2001;121:1428–1436.
67. Yacyshyn BR, Bowen-Yacyshyn MB, Jewell L, et al. A placebo-controlled trial of ICAM-1 antisense oligonucleotide in the treatment of Crohn's disease. Gastroenterology 1998;114:1133–1142.
68. Schreiber S, Nikolaus S, Malchow H, et al. Absence of efficacy of subcutaneous antisense ICAM-1 treatment of chronic active Crohn's disease. Gastroenterology 2001;120:1339–1346.
69. Yacyshyn BR, Chey WY, Goff J, et al. Double blind, placebo controlled trial of the remission inducing and steroid sparing properties of an ICAM-1 antisense oligodeoxynucleotide, alicaforsen (ISIS 2302), in active steroid dependent Crohn's disease. Gut 2002;51:30–36.
70. van Deventer SJ, Tami JA, Wedel MK. A randomised, controlled, double blind, escalating dose study of alicaforsen enema in active ulcerative colitis. Gut 2004;53:1646–1651.
71. von Andrian UH, Engelhardt B. Alpha4 integrins as therapeutic targets in autoimmune disease. N Engl J Med 2003;348:68–72.
72. Ghosh S, Goldin E, Gordon FH, et al. Recombinant humanized antibody to a4 integrin (Natalizumab) in the treatment of active Crohn's disease. N Engl J Med 2003;348:24–321.
73. Sandborn WJ, Colombel JF, Enns R, et al. Natalizumab induction and maintenance therapy for Crohn's disease. N Engl J Med 2005;353:1912–1925.
74. Targan SR, Feagan BG, Fedorak RN, et al. Natalizumab for the treatment of active Crohn's disease: results of the ENCORE Trial. Gastroenterology 2007;132:1672–1683.
75. Gordon FH, Hamilton MI, Donoghue S, et al. A pilot study of treatment of active ulcerative colitis with natalizumab, a humanized monoclonal antibody to alpha-4 integrin. Aliment Pharmacol Ther 2002;16:699–705.
76. Miller DH, Khan OA, Sheremata WA, et al. A controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med 2003;348:15–23.
77. Yednock TA, Cannon C, Fritz LC, et al. Prevention of experimental autoimmune encephalomyelitis by antibodies against $\alpha 4\beta 1$ integrin. Nature 1992;356:63–66.
78. Van Assche G, Van Ranst M, Sciot R, et al. Progressive multifocal leukoencephalopathy after natalizumab therapy for Crohn's disease. N Engl J Med 2005;353:362–368.
79. Yousry TA, Major EO, Ryschkewitsch C, et al. Evaluation of patients treated with natalizumab for progressive multifocal leukoencephalopathy. N Engl J Med 2006;354:924–933.
80. Laroni A, Bedognetti M, Uccelli A, et al. Association of melanoma and natalizumab therapy in the Italian MS population: a second case report. Neurol Sci 2011;32:181–182.
81. Ismail A, Kemp J, Sharrack B. Melanoma complicating treatment with natalizumab (tysabri) for multiple sclerosis. J Neurol 2009;256:1771–1772.
82. Takazoe M, Watanabe M, Kawaguchi T, et al. Oral alpha-4 integrin inhibitor (AJM300) in patients with active Crohn's disease—a randomized, double-blind, placebo-controlled trial. Gastroenterology 2009;136:A181 (Abstract S1066).
83. Major EO. Progressive multifocal leukoencephalopathy in patients on immunomodulatory therapies. Annu Rev Med 2010;61:35–47.
84. Feagan BG, Greenberg GR, Wild G, et al. Treatment of ulcerative colitis with a humanized antibody to the alpha4beta7 integrin. N Engl J Med 2005;352:2499–2507.
85. Feagan BG, Greenberg GR, Wild G, et al. Treatment of active Crohn's disease with MLN0002, a humanized antibody to the alpha4beta7 integrin. Clin Gastroenterol Hepatol 2008;6:1370–1377.
86. Vermeire S, Ghosh S, Panes J, et al. Safety and efficacy of PF-00547,659, a fully human anti-MAdCAM antibody, in ulcerative colitis. Results of a first in human study. Gastroenterology 2009;136:A181 (Abstract S1066).
87. Walters MJ, Wang Y, Lai N, et al. Characterization of CCX282-B, an orally bioavailable antagonist of the CCR9 chemokine receptor, for treatment of inflammatory bowel disease. J Pharmacol Exp Ther 2010;335:61–69.
88. Eksteen B, Adams DH. GSK-1605786, a selective small-molecule antagonist of the CCR9 chemokine receptor for the treatment of Crohn's disease. IDrugs 2010;13:472–781.
89. Hadeiba H, Sato T, Habtezion A, et al. CCR9 expression defines tolerogenic plasmacytoid dendritic cells able to suppress acute graft-versus-host disease. Nat Immunol 2008;9:1253–1260.
90. Wendland M, Czeloth N, Mach N, et al. CCR9 is a homing receptor for plasmacytoid dendritic cells to the small intestine. Proc Natl Acad Sci U S A 2007;104:6347–6352.

Supplementary Table 1. Currently Used Immunomodulatory Therapies for Inflammatory Bowel Disease That Do Not Primarily Target Leukocyte Homing Receptors: Classic Anti-inflammatory/Immunosuppressive Drugs[a]

Pharmacological agent		Indication	Advantages	Disadvantages/side effects	References
Classic anti-inflammatory/ immunosuppressive drugs					
Aminosalicylates	Sulphasalazine Mesalamine	Mild-to-moderate UC and CD (?)	Availability of oral and topical formulations selected principally on the basis of disease location	Maintenance of remission controversial in CD Therapeutic efficacy of mesalamine can depend on mucosal concentration	1, 2
Corticosteroids		Moderated to-severe UC and CD	Available in topical formulations Suppress active inflammation in the acute setting	Side effect profile does not allow long-term treatment Possible high relapse rate	3
Immunomodulation and/or inhibition of lymphocyte activation					
Thiopurines	Azathioprine 6-mercaptopurine	Mild-to-moderate UC and CD	Effective maintenance immunosuppressant agents indicated for steroid-dependent patients	Slow onset of action and potential serious adverse events and toxicity (toxic hepatitis, pancreatitis and lymphopenia, opportunistic infections)	4
Cyclosporin A		Severe UC and CD refractory to conventional therapy	Rapidly acting therapeutic agent	Use restricted to hospitalized patients Potential risks of hypertension, nephrotoxicity, electrolyte imbalance, encephalopathy, tremors, myelosuppression, opportunistic infections, and seizures	5, 6
Methotrexate		Steroid-dependent CD	Maintenance of remission after successful induction	Potential myelosuppression, hepatotoxicity, and teratogenic and abortigenic effects	7, 8

[a]Inhibitors of leukocyte traffic molecules were summarized separately in Table 2.

Supplementary Table 2. Currently Used Immunomodulatory Therapies for Inflammatory Bowel Disease That Do Not Primarily Target Leukocyte Homing Receptors: Inhibitors of Proinflammatory Cytokines

Target	Biological agent	Indication	Mechanism	Advantages	Disadvantages/ side effects	References
TNF-α blockers	Infliximab	Moderate-to-severe UC/CD refractory to conventional therapy	Chimeric mAb targeting human TNF-α. Binds soluble bioactive TNF in the intestinal mucosa neutralizing its effect. Binds to membrane-bound TNF, leading to T-cell apoptosis	Long-term clinical benefit; permits tapering of corticosteroids; effective in the treatment of extraintestinal IBD manifestations	Drug-induced lupus acute infusion reactions; delayed hypersensitivity reactions, demyelination; limited but real risk of infections, lymphoma, cardiac failure	9–12
	Adalimumab	CD refractory to conventional therapy	Fully human IgG1 anti–TNF-α mAb	Well-tolerated; decrease in immunogenicity compared to infliximab	Injection site reactions	13

mAb, monoclonal antibody.

Supplementary Table 3. Promising Immunomodulatory Therapies for Inflammatory Bowel Disease: Inhibitors of Proinflammatory Cytokines

Target	Biological agent	Indication	Mechanism	Clinical phase	Advantages	Disadvantages/ side effects	References
TNF-α blockers	RDP58 (delmitide acetate)	Mild-to-moderate active UC	Protease resistant decapeptide; inhibits synthesis of pro-inflammatory cytokines (TNF, IFN-γ, IL-2, and IL-12) by blocking the formation of the MyD88-IL-1 receptor-associated kinase (IRAK)-TRAF6 cell signaling protein complex	Phase III/IIIb	Oral solution; no systemic bioavailability; not immunogenic	No major adverse events reported	14
	Certolizumab pegol	CD	Humanized TNF-α Fab′ mAb fragment linked to polyethylene glycol	Phase III (only in United States)	Increased drug plasma half-life	Modest improvement in response rates; risk of infections	15, 16

IFN, interferon; IL, interleukin; mAb, monocolonal antibody.

Supplementary Table 4. Unsuccessful/Unproven Immunomodulatory Therapies in Inflammatory Bowel Disease

Target	Biologic agent	Indication	Mechanism	Clinical phase	Advantages	Disadvantages/ side effects	References
Inhibitor of T-cell activation							
	IDEC-131	CD	Anti-CD40 ligand	Phase II discontinued		Thromboembolism	17
Anti-CD4 therapy	cM-T412	CD/UC	Anti-CD4 depleting mAb	Phase I discontinued	Short-term clinical improvement/remission	CD4 lymphopenia	18, 19
	MAX.16H5 and B-F5	CD/UC	Anti-CD4 non-depleting mAb	Phase I discontinued	Clinical improvement in UC	CD4 lymphopenia	20
Anti-CD3 therapy	Visilizumab (UhM291)	Severe and refractory UC	Humanized IgG2 Anti-CD3e mAb; induces T-cell apoptosis and enhances IL-10 secretion	Phase III suspended	Clinical response observed in the majority of patients	Dose-limiting toxicities; transient decrease in T-lymphocyte counts; liver injury; cytokine-release symptoms	21, 22
Anti-inflammatory cytokines							
	rhIL-10	Refractory CD	Down-regulates lymphocytes' activation	Failed phase II/III	Safe and well-tolerated	Ineffective even in oral formulation	23
	rhIL-11	Mild-to-moderate active CD	Enhance epithelial integrity	Phase II/III	Subcutaneous administration safe and well-tolerated	Minor injection site reactions	24, 25
Inhibitors of proinflammatory cytokine receptor							
	Tocilizumab	Active CD	Humanized IgG1 monoclonal antibody to IL-6 receptor; increases apoptosis of mononuclear cells	Phase II	Well-tolerated	Efficacy not definitely proven	26
Inhibitors of Th1 polarization							
	Fontolizumab	Moderate-to-severe active CD	Humanized anti-IFN-γ mAb	Phase II	Well-tolerated, with a good safety profile	Efficacy not definitely proven	27, 28
	ABT-874	Active CD	Human anti-IL12/23 p40 mAb	Phase II		Limited clinical response; injection site reactions; antidrug antibodies development	29
Inhibitors of T–cell proliferation							
Anti−IL-2 receptor therapy	Daclizumab	UC	Humanized IgG1 anti–IL-2 receptor (CD25) mAb	Phase II	Clinical benefit	Efficacy not definitely proven	
	Basiliximab	UC	Chimeric monoclonal anti-CD25 mAb	Phase II	Clinical remission in combination with steroid treatment	Efficacy not definitely proven	
Growth hormone and growth factors							
	Somatropin (growth hormone)	CD	Stimulates production of insulin-like growth factor 1; trophic for intestinal mucosa	Phase II	Clinical benefit with decreased disease score; improved diarrhea and overall well-being	Efficacy not definitely proven	30
	Keratinocyte growth factor (repifermin, KGF-2)	Active UC	Stimulates epithelial proliferation and repair through activation of PI3K/AKT and MAPK pathway	Phase II	Safe and well-tolerated	No adverse effects; no proven efficacy over placebo	
	Epidermal growth factor	UC	Induces epithelial growth through activation of PI3K/AKT and MAPK pathway	Phase II	Highly efficacious in patients with active distal UC	Risk of malignant transformation in predisposed patients	31
	Sargramostim (recombinant human GM-CSF)	Steroid-dependent CD	Activates Jak/STAT pathway, PI3k/AKT, and MAPK; immunostimulant effect on neutrophils	Phase II	Well-tolerated	No clear benefit over placebo; irritation at the injection site; bone pain; dyspnea	32, 33
	Filgrastim (recombinant human G-CSF)	CD	Immunostimulant effect on neutrophils; prevents apoptosis in epithelial cells	Phase II	Clinical remission and mucosal healing	Transient bone pain	34

G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3 kinase; rh, recombinant human; STAT, signal transducer and activation of transcription.

Supplementary Table 1 References

1. Higgins PD, Rubin DT, Kaulback K, et al. Systematic review: impact of non-adherence to 5-aminosalicylic acid products on the frequency and cost of ulcerative colitis flares. Aliment Pharmacol Ther 2009;29:247–257.
2. Kane SV, Cohen RD, Aikens JE, et al. Prevalence of nonadherence with maintenance mesalamine in quiescent ulcerative colitis. Am J Gastroenterol 2001;96:2929–2933.
3. Irving PM, Gearry RB, Sparrow MP, et al. Review article: appropriate use of corticosteroids in Crohn's disease. Aliment Pharmacol Ther 2007;26:313–329.
4. Chevaux JB, Peyrin-Biroulet L, Sparrow MP. Optimizing thiopurine therapy in inflammatory bowel disease. Inflamm Bowel Dis 2010 Oct 14. [Epub ahead of print].
5. Maser EA, Deconda D, Lichtiger S, et al. Cyclosporine and infliximab as rescue therapy for each other in patients with steroid-refractory ulcerative colitis. Clin Gastroenterol Hepatol 2008;6:1112–1116.
6. Cacheux W, Seksik P, Lemann M, et al. Predictive factors of response to cyclosporine in steroid-refractory ulcerative colitis. Am J Gastroenterol 2008;103:637–642.
7. Wahed M, Louis-Auguste JR, Baxter LM, et al. Efficacy of methotrexate in Crohn's disease and ulcerative colitis patients unresponsive or intolerant to azathioprine/mercaptopurine. Aliment Pharmacol Ther 2009;30:614–620.
8. Nathan DM, Iser JH, Gibson PR. A single center experience of methotrexate in the treatment of Crohn's disease and ulcerative colitis: a case for subcutaneous administration. J Gastroenterol Hepatol 2008;23:954–958.
9. Sands BE, Anderson FH, Bernstein CN, et al. Infliximab maintenance therapy for fistulizing Crohn's disease. N Engl J Med 2004;350:876–885.
10. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med 2005;353:2462–2476.
11. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med 2010;362:1383–1395.
12. Diak P, Siegel J, La Grenade L, et al. Tumor necrosis factor alpha blockers and malignancy in children: forty-eight cases reported to the Food and Drug Administration. Arthritis Rheum 2010;62:2517–2524.
13. Sandborn WJ, Hanauer SB, Rutgeerts P, et al. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. Gut 2007;56:1232–1239.
14. Travis S, Yap LM, Hawkey C, et al. RDP58 is a novel and potentially effective oral therapy for ulcerative colitis. Inflamm Bowel Dis 2005;11:713–719.
15. Schreiber S, Khaliq-Kareemi M, Lawrance IC, et al. Maintenance therapy with certolizumab pegol for Crohn's disease. N Engl J Med 2007;357:239–250.
16. Sandborn WJ, Feagan BG, Stoinov S, et al. Certolizumab pegol for the treatment of Crohn's disease. N Engl J Med 2007;357:228–238.
17. Dumont FJ. IDEC-131. IDEC/Eisai. Curr Opin Investig Drugs 2002;3:725–734.
18. Emmrich J, Seyfarth M, Fleig WE, et al. Treatment of inflammatory bowel disease with anti-CD4 monoclonal antibody. Lancet 1991;338:570–571.
19. Canva-Delcambre V, Jacquot S, Robinet E, et al. Treatment of severe Crohn's disease with anti-CD4 monoclonal antibody. Aliment Pharmacol Ther 1996;10:721–727.
20. Stronkhorst A, Radema S, Yong SL, et al. CD4 antibody treatment in patients with active Crohn's disease: a phase 1 dose finding study. Gut 1997;40:320–327.
21. Plevy S, Salzberg B, Van Assche G, et al. A phase I study of visilizumab, a humanized anti-CD3 monoclonal antibody, in severe steroid-refractory ulcerative colitis. Gastroenterology 2007;133:1414–1422.
22. Baumgart DC, Targan SR, Dignass AU, et al. Prospective randomized open-label multicenter phase I/II dose escalation trial of visilizumab (HuM291) in severe steroid-refractory ulcerative colitis. Inflamm Bowel Dis 2010;16:620–629.
23. Braat H, Peppelenbosch MP, Hommes DW. Interleukin-10-based therapy for inflammatory bowel disease. Expert Opin Biol Ther 2003;3:725–731.
24. Sands BE, Winston BD, Salzberg B, et al. Randomized, controlled trial of recombinant human interleukin-11 in patients with active Crohn's disease. Aliment Pharmacol Ther 2002;16:399–406.
25. Herrlinger KR, Witthoeft T, Raedler A, et al. Randomized, double blind controlled trial of subcutaneous recombinant human interleukin-11 versus prednisolone in active Crohn's disease. Am J Gastroenterol 2006;101:793–797.
26. Ding C, Jones G. Anti-interleukin-6 receptor antibody treatment in inflammatory autoimmune diseases. Rev Recent Clin Trials 2006;1:193–200.
27. Hommes DW, Mikhajlova TL, Stoinov S, et al. Fontolizumab, a humanised anti-interferon gamma antibody, demonstrates safety and clinical activity in patients with moderate to severe Crohn's disease. Gut 2006;55:1131–1137.
28. Reinisch W, Hommes DW, Van Assche G, et al. A dose escalating, placebo controlled, double blind, single dose and multidose, safety and tolerability study of fontolizumab, a humanised anti-interferon gamma antibody, in patients with moderate to severe Crohn's disease. Gut 2006;55:1138–1144.
29. Mannon PJ, Fuss IJ, Mayer L, et al. Anti-interleukin-12 antibody for active Crohn's disease. N Engl J Med 2004;351:2069–2079.
30. Slonim AE, Bulone L, Damore MB, et al. A preliminary study of growth hormone therapy for Crohn's disease. N Engl J Med 2000;342:1633–1637.
31. Sinha A, Nightingale J, West KP, et al. Epidermal growth factor enemas with oral mesalamine for mild-to-moderate left-sided ulcerative colitis or proctitis. N Engl J Med 2003;349:350–357.
32. Dieckgraefe BK, Korzenik JR. Treatment of active Crohn's disease with recombinant human granulocyte-macrophage colony-stimulating factor. Lancet 2002;360:1478–1480.
33. Korzenik JR, Dieckgraefe BK, Valentine JF, et al. Sargramostim for active Crohn's disease. N Engl J Med 2005;352:2193–2201.
34. Korzenik JR, Dieckgraefe BK. An open-labelled study of granulocyte colony-stimulating factor in the treatment of active Crohn's disease. Aliment Pharmacol Ther 2005;21:391–400.