Leukocyte Adhesion Molecules in Animal Models of Inflammatory Bowel Disease

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Abstract: The dysregulated recruitment of leukocytes into the intestine is required for the initiation and maintenance of inflammatory bowel disease (IBD). Several families of molecules regulate the influx of these cells into sites of inflammation. Interference with some of these molecules has already shown efficacy in the clinics and antibodies that target the molecules involved have been approved by the FDA for use in Crohn's disease (CD), multiple sclerosis (i.e., natalizumab), and psoriasis (i.e., efalizumab). Here, we discuss basic aspects of the different families of relevant molecules and compile a large body of preclinical studies that supported the targeting of specific steps of the leukocyte adhesion cascade for therapeutic purposes in colitis and in novel models of CD-like ileitis.

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Key Words: recruitment of leukocytes, adhesion cascade, IBD

Recent advances in our understanding of the fundamental mechanisms of seemingly unrelated diseases, i.e., rheumatoid arthritis (RA), Type 1 diabetes mellitus, psoriasis, multiple sclerosis, and inflammatory bowel disease (IBD), have demonstrated that they share similar pathogenetic mechanisms. These diseases are immune-mediated chronic inflammatory conditions, characterized by inappropriate and sustained recruitment of inflammatory cells into affected tissues, resulting in chronic tissue damage and loss of function.[1–3]

The fate of the inflammatory cells differs according to cell type. Neutrophils migrate to sites of inflammation and undergo apoptosis. Some monocytes remain in inflamed tissues for a few days and others become permanent residents. By contrast, lymphocytes not only migrate but recirculate from the blood into tissues and through the lymphatics back to lymphatic tissues and blood, constantly patrolling for foreign antigens. Once they encounter an antigen in the context of an antigen-presenting cell, naïve T cells proliferate and acquire effector functions, along with a repertoire of surface molecules (i.e., adhesion molecules, cytokine and chemokine receptors) that allow them to recognize counter-receptors/ligands expressed in specific vascular beds. Using these surface molecules, lymphocytes are able to recirculate thousands of times back to areas with a similar microenvironment to where they first encountered their cognate antigen.[4,5] These unique capabilities (i.e., memory acquisition and recirculation) are essential for the perpetuation of chronic inflammatory processes, including IBD. The molecules involved in the recirculation of lymphocytes have therefore attracted a great deal of interest regarding their potential as therapeutic targets. Some of these have crossed from the bench into the clinical arena, being currently in clinical use.[6–8]

In IBD the inflammatory process is characterized by heavy leukocytic infiltration of the intestinal lamina propria (LP), leading to fibrosis and loss of function.[9–11] Lymphocytes that produce cytokines such as IL-12, IFN-γ, tumor necrosis factor-α (TNF-α), IL-23, and IL-17[12] all play an important role in chronic intestinal inflammation.[9–13]

The success of the anti-TNF-α strategy in IBD[14] has led to the systematic study of antiinflammatory cytokines and the development of antibody-based strategies to modulate the overall cytokine balance.[15,16] Unfortunately, the therapeutic efficacy of some of these newer cytokine-targeted therapies (e.g., IL-10, IL-11 blockade) has been limited.[15,16] A neutralizing antibody against the IL-12 p40 subunit, shared by IL-12 and IL-23, has shown promise.[17] Alternative therapies that target other pathways of the chronic inflammatory process may be directed at interfering with lymphocyte recirculation to the intestine by targeting specific adhesion molecules, their ligands, chemokines, or their receptors.[18–20] Using this approach, 2 monoclonal antibodies against integrin α_4 and $\alpha_L\beta_2$ have been approved by the FDA for the treatment of multiple sclerosis (MS), Crohn's disease (CD), and psoriasis (i.e., natalizumab, efalizumab).[6,18] However, many of the basic mechanisms that account for their clinical efficacy remain to be elucidated. This limited knowledge has likely contributed

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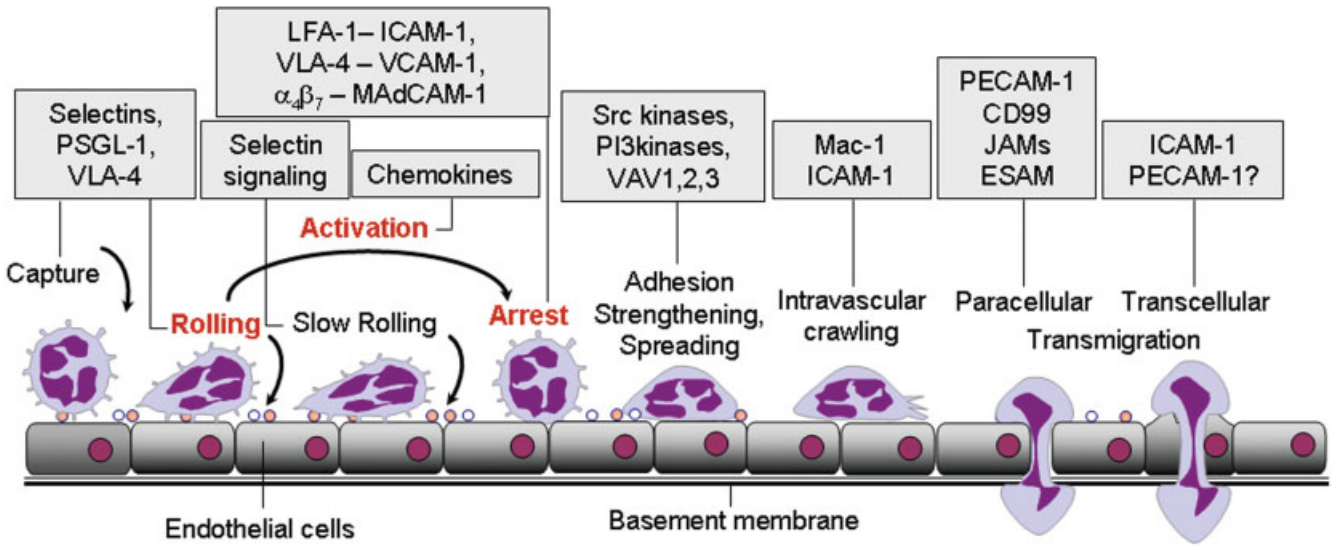

FIGURE 1. The original steps of the leukocyte adhesion cascade are shown in red: rolling, mediated by selectins, activation, mediated by chemokines, and arrest, mediated by integrins. This has been expanded to include additional steps: capture (or tethering), slow rolling, adhesion strengthening and spreading, intravascular crawling, and paracellular and transcellular transmigration. Crucial molecules involved are indicated in boxes. ESAM, endothelial cell-selective adhesion molecule; ICAM-1, intercellular adhesion molecule 1; JAM, junctional adhesion molecule; LFA-1, lymphocyte function-associated antigen 1 (also known as $\alpha_L\beta_2$-integrin); Mac-1, macrophage antigen 1; MAdCAM-1, mucosal vascular addressin cell-adhesion molecule 1; PSGL-1, P-selectin glycoprotein ligand 1; PECAM-1, platelet/endothelial-cell adhesion molecule 1; PI3Ki ases, phosphoinositide 3-kinases; VCAM-1, vascular cell-adhesion molecule 1; VLA-4, very late antigen 4 (also known as $\alpha_4\beta_1$-integrin).[24]

to the occurrence of serious adverse events in clinical practice.[21,22]

LEUKOCYTE ADHESION CASCADE

Leukocytes primarily migrate from the blood into the tissues across the walls of postcapillary venules. Surface molecules on specialized venular endothelial cells play a crucial role. These adhesion molecules not only serve as mechanical anchors, but also confer tissue specificity to the recruitment process through their selective patterns of expression by vascular beds.[23] Myeloid cells and lymphocytes share some of the steps in the adhesion cascade, but there are also significant differences.[24] This review focuses primarily on lymphocyte recruitment to the intestine and how this process has been targeted for therapeutic purposes in animal models of colitis and ileitis that mimic aspects of either UC or CD, respectively.

Leukocyte Recruitment

Several major classes of leukocyte adhesion molecules are involved in leukocyte recruitment, including the selectins and their glycoprotein ligands, integrins and immunoglobulin-superfamily molecules. They are all type I transmembrane glycoproteins that span the cell membrane only once. The structural and functional aspects of these adhesion molecules have been extensively discussed elsewhere.[25–28]

The process of leukocyte recruitment to a site of inflammation encompasses the engagement and efficient arrest of leukocytes onto the vascular endothelium and their subsequent transmigration.[4,23,29] This sequence is composed of several major steps: capture, rolling, activation, and firm adhesion (Fig. 1).

Capture

Capture is defined as the formation of the first molecular bond or tether between the circulating leukocyte and the vascular endothelium. Close proximity between the cells is required. Capture is distinguishable from stable rolling and is mediated by L- and P-selectins. In inflamed venules in vivo, leukocyte attachment also involves other mechanisms, such as secondary capture through leukocyte–leukocyte interaction.[30,31] Most neutrophils start rolling as they exit capillaries,[32] whereas naïve lymphocytes capture in high endothelial venules (HEV) of peripheral lymph nodes (PLN) and other lymphoid tissues.[5] Little is known about capture of effector/memory lymphocytes, although selectins and CD44 may be involved.[33,34]

Rolling

If leukocyte capture is followed by the formation of new molecular bonds before the initial molecular bonds dissociate, a stable rolling movement is established.[35,36] This is a flow-driven downstream movement of the cell, during which it is in continuous contact with the vessel wall. Naïve lymphocyte rolling in HEV is mainly mediated by lympho-

cyte L-selectin binding to sulfated carbohydrate-containing ligands expressed on endothelial cells. Rolling has at least two distinct consequences for the cell: 1) it facilitates stable leukocyte arrest (firm adhesion), and 2) it drastically reduces leukocyte velocity (to between 1 and 100 μm/s), increasing the duration of exposure to the endothelial surface and to chemokines and other activating signals present there. Neutrophils arrest after gradually slowing down,[37] whereas naïve lymphocytes arrest immediately upon encountering chemokines.[38] Effector T cells can arrest through the chemokine receptor CXCR3 in vitro, but the molecules involved in arrest in vivo remain unknown.[39]

Firm Adhesion

Activation of the rolling lymphocyte can be triggered by the binding of a chemokine to a heptahelical transmembrane receptor on the leukocyte surface and can result in firm adhesion.[23,40] In flow chamber systems, this process is exceptionally rapid.[41,42] Prior studies, focused on lymphocyte function-associated antigen (LFA)-1 have shown that firm adhesion requires binding of integrin receptors in their active conformation to their endothelial ligands.[43] Both $\alpha_4\beta_1$ (VLA-4) and $\alpha_4\beta_7$ integrins have been shown to mediate firm adhesion to their respective ligands, vascular cell adhesion molecule (VCAM)-1 and mucosal addressin cell adhesion molecule (MAdCAM)-1.[44,45]

Families of Adhesion Molecules Involved in Leukocyte Recruitment

Selectins

The selectins are a family of transmembrane mammalian lectins expressed on the surface of leukocytes (L-selectin), endothelial cells (P-, E-selectins), and platelets (P-selectin).[27] The selectins contain an N-terminal extracellular domain with structural homology to calcium-dependent lectins, followed by a domain homologous to epidermal growth factor, and 2 to 9 consensus repeats (CR), similar to sequences found in complement regulatory proteins (Fig. 2). Each selectin inserts into the cell membrane via a hydrophobic transmembrane domain and possesses a short cytoplasmic tail. Each of the 3 selectins can independently mediate leukocyte rolling, given the appropriate conditions. The 3 selectins share ≈50% sequence homology among the extracellular portions of the molecule, but there is no significant homology in the transmembrane and cytoplasmic domains.[27,46]

All 3 selectins are involved in the trafficking of granulocytes and lymphocytes to sites of inflammation. However, the role of each selectin differs in the physiological sequence of events, and the importance of each selectin varies in different models of inflammation. Importantly, L-selectin is essential for the homing of lymphocytes to peripheral lymph nodes,[47] and plays an accessory role in lymphocyte homing to Peyer's patches (PP), a component of the gut-associated

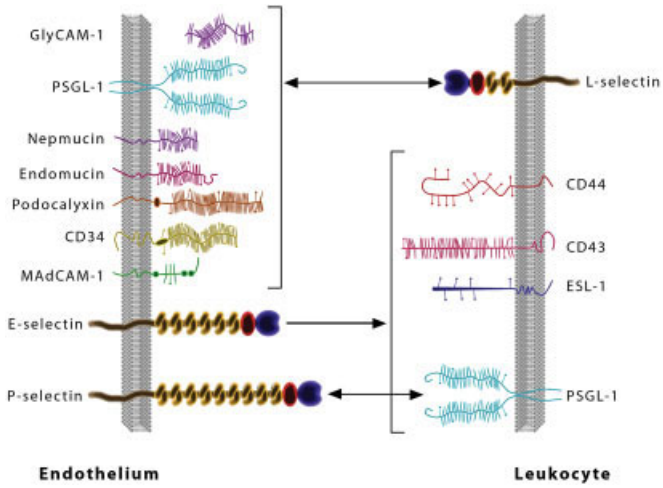

FIGURE 2. Selectins and their ligands. During the inflammatory response, leukocyte adhesion to endothelial cells is controlled by the binding of selectins to complementary carbohydrate ligands. All known selectin ligands relevant for lymphocyte trafficking are transmembrane glycoproteins, which present oligosaccharide structures to the selectins. Transient bond formations between the selectins and their ligands mediate the early steps of the adhesion cascade. All 3 selectins can recognize glycoproteins presenting the tetrasaccharide sialyl-LewisX (sialyl-CD15). This tetrasaccharide is found on all circulating myeloid cells and some activated T cells. It is composed of sialic acid, galactose, fucose, and N-acetyl-galactosamine. It is unclear how selectins achieve specific interactions with ligands, given this common carbohydrate recognition.

lymphatic tissue.[47] HEV in these tissues constitutively express functional ligands for L-selectin on their surface. The importance of L-selectin in lymphocyte homing is emphasized by the decreased size of lymph nodes and a delay in lymphocyte homing to Peyer's patches in L-selectin-deficient mice.[47] In PP, L-selectin synergizes with β_7 integrins, since absence of both L-selectin and integrins completely abrogates lymphocyte homing to these structures.[48]

T-cell activation results in proteolytic shedding of L-selectin.[49,50] This results in pattern of surface molecule expression ($CD44^{high}$ $L\text{-}selectin^{low}$) a characteristic of effector memory T cells that recirculate to effector sites, while limiting their traffic through secondary lymphoid organs.[5] However, subpopulations of memory cells (i.e., central memory, T_{CM})[51] retain or reexpress L-selectin to recirculate through inductive sites and potentially to tertiary lymphoid organs present at sites of chronic inflammation, where functional ligands for L-selectin are expressed.

P-selectin

P-selectin (CD62P) contains 9 consensus repeats (CR) and extends ≈40 nm from the endothelial surface (Fig. 2). P-selectin is constitutively synthesized by megakaryocytes and endothelial cells, but is not expressed on the surface of resting platelets or endothelial cells. Rather, it is stored in

secretory vesicles called Weibel–Palade bodies in endothelial cells, and α-granules in platelets.[52,53] These granules are triggered to fuse with the plasma membrane by activators such as histamine, thrombin, complement C5a, calcium ionophores, and adenosine diphosphate (ADP), resulting in expression of P-selectin on the cell surface.[54] Maximal levels of P-selectin are detected within minutes at the cell surface, followed by rapid internalization and degradation or recycling.[52] In the mouse, activation of endothelial cells by cytokines prolongs the surface expression of P-selectin.[55–57] Yet in humans, expression of P-selectin is not induced by cytokine stimulation, possibly a result of structural differences in the P-selectin promoter.[58]

The transient interactions between P-selectin and P-selectin glycoprotein ligand (PSGL)-1 allow leukocytes to roll along the venular endothelium. P- and E-selectins tend to have overlapping functions in TNF-α-stimulated venules. Indeed, in mice deficient for P-selectin it is necessary to block E-selectin function to significantly reduce rolling, and in E-selectin knockouts an antibody against P-selectin must be introduced to reduce rolling. Correspondingly, no leukocyte rolling is observed in E-selectin/P-selectin double-deficient mice treated with TNF-α.[59,60] Nonetheless, although P- and E-selectins seem to have redundant functions: observations of rolling flux fractions and rolling velocities indicate that under physiological conditions, P-selectin is responsible for early rolling, while E-selectin allows slow rolling and adhesion.[37,61] In mice deficient for P-selectin, rolling is absent initially[62] but returns after 1–2 hours.[63] Endothelial cell expression of P-selectin is elicited within minutes by inflammatory mediators such as histamine, thrombin, or phorbol esters. The expression is short-lived, reaching its peak after only 10 minutes. In mice, additional synthesis of P-selectin is brought about within 2 hours by cytokines such as IL-1 or TNF-α.[55–57]

E-selectin

E-selectin is expressed by endothelial cells after activation by IL-1 or TNF-α,[64,65] but unlike P-selectin it is not expressed by platelets. On the surface of cultured endothelial cells it is only expressed transiently for a period of 2–6 hours. Subsequently, E-selectin can either be re-internalized or shed from the endothelial surface.[66] In vivo, the expression of E-selectin appears to be more prolonged in some organs[67] and is constitutive in skin microvessels.[68,69] As discussed earlier, the role of E-selectin during rolling is partially redundant with that of P-selectin. E-selectin-deficient mice have a reduced number of firmly adherent leukocytes in response to local chemoattractants[70] or cytokine stimulation,[71] thus E-selectin participates in the conversion of rolling to firm adhesion. E-selectin ligation of PSGL-1 triggers neutrophil activation through spleen tyrosine kinase (Syk).[72] E-selectin is of particular importance in skin inflammation, as it supports the recruitment of skin-specific T lymphocytes.[73] The ligand(s) responsible for E-selectin-mediated rolling interactions include PSGL-1, CD44, and E-selectin ligand-1 (ESL-1) (Fig. 2).[74–76] In addition to glycoproteins, glycolipids can also support E-selectin-dependent rolling in vitro.[77] E-selectin mediates much slower rolling than P-selectin, because it triggers partial LFA-1 activation.[72] These rolling velocities range between less than 5 μm/s[61,78] and 15 μm/s.[70] The molecular dissociation rate or off-rate of E-selectin is very similar to that of P-selectin.[79] However, the velocity of E-selectin-mediated rolling is remarkably invariant with wall shear rate, presumably because E-selectin engagement triggers LFA-1 activation.[72] A role of E-selectin and its ligands has been demonstrated for lymphocyte homing, particularly to skin. Cutaneous-homing effector/memory T cells express a carbohydrate epitope (i.e., cutaneous associated lymphocyte antigen [CLA]) which decorates glycoproteins such as PSGL-1 and CD43 and is associated with E-selectin ligand activity.[80]

L-selectin

L-selectin, a 74–100-kDa molecule also known as CD62L, is constitutively expressed at the tips of microfolds or microvilli on granulocytes, monocytes, and most circulating lymphocytes. Lymphocytes preferentially express it when in the naïve state. In addition, it is expressed by central memory T cells,[51] and most natural killer cells,[27] as well as by many immature hematopoietic cells in the bone marrow. L-selectin expression on neutrophils correlates negatively with the age of the individual cell, suggesting that neutrophils may lose L-selectin as they mature in the bone marrow[81] and as they age in the bloodstream.[82] Although most lymphocytes in the cortex of secondary lymphoid organs express L-selectin, the activated cells in the germinal centers do not.[27] This indicates that expression of L-selectin is at least transiently reduced from activated lymphocytes. L-selectin is proteolytically cleaved from the surface of leukocytes after activation by a variety of stimuli.[49,83–85] For neutrophils, activating agents that induce L-selectin shedding include inflammatory chemokines such as IL-8, complement factors such as C5a, bacterial peptides such as formyl-methionyl-leucyl-phenylalanine (fMLP), and lipid mediators such as platelet activating factor (PAF) or leukotriene B4 (LTB4).[86] In vitro, lymphocytes shed L-selectin when they are stimulated with phorbol esters or by crosslinking of CD3.[87] In vivo, soluble L-selectin is present in the plasma at high concentrations,[88] most of which is derived from lymphocytes.[89] L-selectin is responsible for naïve lymphocyte homing to PLN and MLN, but is also expressed by central memory T cells, which may play a role in the maintenance of IBD[90] and other chronic inflammatory conditions.

Selectin Ligands

PSGL-1

PSGL-1 is the best-characterized selectin ligand (Fig. 2).[91,92] It is a type 1 surface dimeric glycoprotein that is expressed on the microprocesses of virtually all leukocytes.

PSGL-1 is also expressed in certain endothelial cells within the walls of the small intestine,[93] and inflamed colon,[94] as well as in cultured umbilical vein and microvascular endothelial cells.[95,96] Certain posttranslational modifications are necessary for PSGL-1 to function as a selectin ligand, including tyrosine sulfation,[96,97,98] sialylation,[99] decoration with core 2 oligosaccharides,[100] and, perhaps most importantly, fucosylation.[98] Fucosylation is regulated by the specific, inducible fucosyl transferase (FTVII), which plays a key role in the generation of functional selectin ligands.[100–103] T-helper 1 (Th1)-polarized T cells preferentially express functional P- and E-selectin ligands.[104–107] Indeed, the expression of FTVII is modulated by differentiation into Th1 and Th2 phenotypes.[33] FTVII is constitutively expressed by neutrophils, monocytes, and eosinophils.[101,102] The trafficking of neutrophils is severely restricted in mice that lack FTVII,[108] although unlike mice that lack both E- and P-selectin, these mice do not develop spontaneous disease.[59,60] The importance of selectin ligands is dramatically illustrated by a rare human disease, Leukocyte Adhesion Deficiency-II (LAD-II). A fucosylation defect in these patients renders selectin ligands inactive, causing an inability of leukocytes to roll.[109] These patients have significant inflammatory pathology.[110] The binding of PSGL-1 to P-selectin and the functional implications thereof have been demonstrated in vivo.[111,112] PSGL-1 also binds L-selectin,[113,114] and appears to be responsible for most,[115] though not all,[116] of the interactions between flowing and already adherent leukocytes. Although all lymphocytes express PSGL-1, this molecule does not only function as a selectin ligand, but also binds the chemokines CCL19 and CCL21.[117]

Other Selectin Ligands

Other glycoproteins serve as ligands for L-selectin, including glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1),[118] nepmucin, endomucin,[28] CD34,[119] and podocalyxin.[120] Most L-selectin ligands expressed by high endothelial venules contain sulfated determinants that are identified using the carbohydrate-binding monoclonal antibody MECA-79.[121,122] MECA-79 binds to a sulfation-dependent epitope and can block L-selectin binding.[121] This epitope overlaps with sialyl 6-sulfo Lewis X, an L-selectin recognition determinant.[123] Both PNAd and sialyl 6-sulfo Lewis X are absent in mice deficient for 2 N-acetylglucosamine-6-O-sulfotransferases (GlcNAc6ST-1 and GlcNAc6ST-2), resulting in reduced lymphocyte homing and adhesion in HEV.[123,124] MECA-79 binding requires sulfation. Recent studies have identified the molecular structure of its epitope as an extended core 1 structure containing GlcNAc-6-SO4 on O-linked glycans.[125] The enzymes HECGlcNAc-6-sulfotransferases (HEC-6ST also known as GST, L-selectin ligand sulfotransferase [LSST]) are responsible for posttranslational modifications that are essential for L-selectin binding. HEC-6ST expression is highly restricted to HEV, and in humans and mice a sulfotransferase with restricted expression to the intestine has been identified.[126] Little is known about its role in inflammation, but two recent studies have demonstrated its induction by TNF in human bronchial mucosa and lymphotoxin in an animal model of lymphoid neogenesis in the pancreas.[127,128]

Another ligand for L-selectin is mucosal addressin adhesion molecule-1 (MAdCAM-1), expressed on the surface of high endothelial venules of Peyer's patches and mucosal lymph nodes.[129,130] MAdCAM-1 isolated from mesenteric lymph nodes of young mice supports L-selectin-dependent lymphocyte rolling in a flow chamber assay.[131] A role for L-selectin in lymphocyte rolling in Peyer's patch high endothelial venules has also been demonstrated by intravital microscopy, but it is not clear whether all L-selectin ligand activity in this system is attributable to MAdCAM-1.[44,48] The phenotype of MAdCAM-1-deficient mice is identical to that of B7-integrin deficient mice, suggesting that the role of MAdCAM-1 as an L-selectin ligand might be of limited consequence in vivo.

For E-selectin, a 250-kDa potential ligand was precipitated from bovine γδ T cells,[132] whereas on myeloid cells, PSGL-1, CD44, and E-selectin ligand-1 (ESL-1), a molecule with homology to fibroblast growth factor receptor, are E-selectin ligands.[74,75] In addition, CD43 might be a relevant ligand for E-selectin on skin-homing activated T cells.[133,134]

Strategies to Target Selectins Therapeutically

Several selectin inhibitors have been developed and tested in preclinical models and clinical trials. Carbohydrate-based selectin inhibitors of the sialyl Lewis^x type, which inhibit all three selectins at high concentrations, have unfavorable pharmacokinetics, low affinity, and short half lives.[135] Drug candidates within this family (e.g., OC229648, Efomycine, and CY1503) have been tested in preclinical models of peritonitis, psoriasis, and ischemia-reperfusion injury. Antibodies to selectins including antibodies that block more than one selectin have been developed and humanized. Protein Design Laboratories (www.pdl.com) has tested a humanized version of an anti-L-selectin antibody (HuDREG-55) in patients with trauma and in psoriasis. An antibody against both P- and E-selectin (Hu EP5C7) has been tested in a baboon stroke model, while specific antibodies against E- (i.e., CDP850) and P-selectin (CY1747) have been tested in psoriasis and preclinically in a model of ischemia-reperfusion injury. A recombinant truncated form of a PSGL-1-immunoglobulin fusion protein has shown promise as a selectin inhibitor[136] (mainly aimed at P- and L-selectin) (www.wyeth-.com). This molecule is currently in clinical trials for liver and renal transplantation (http://www.ysthera.com/news/070502.html). Small-molecule inhibitors of selectins known as glycomimetics have also been developed.[137] A small-molecule inhibitor of selectin function (i.e., TBC-1269, Bi-

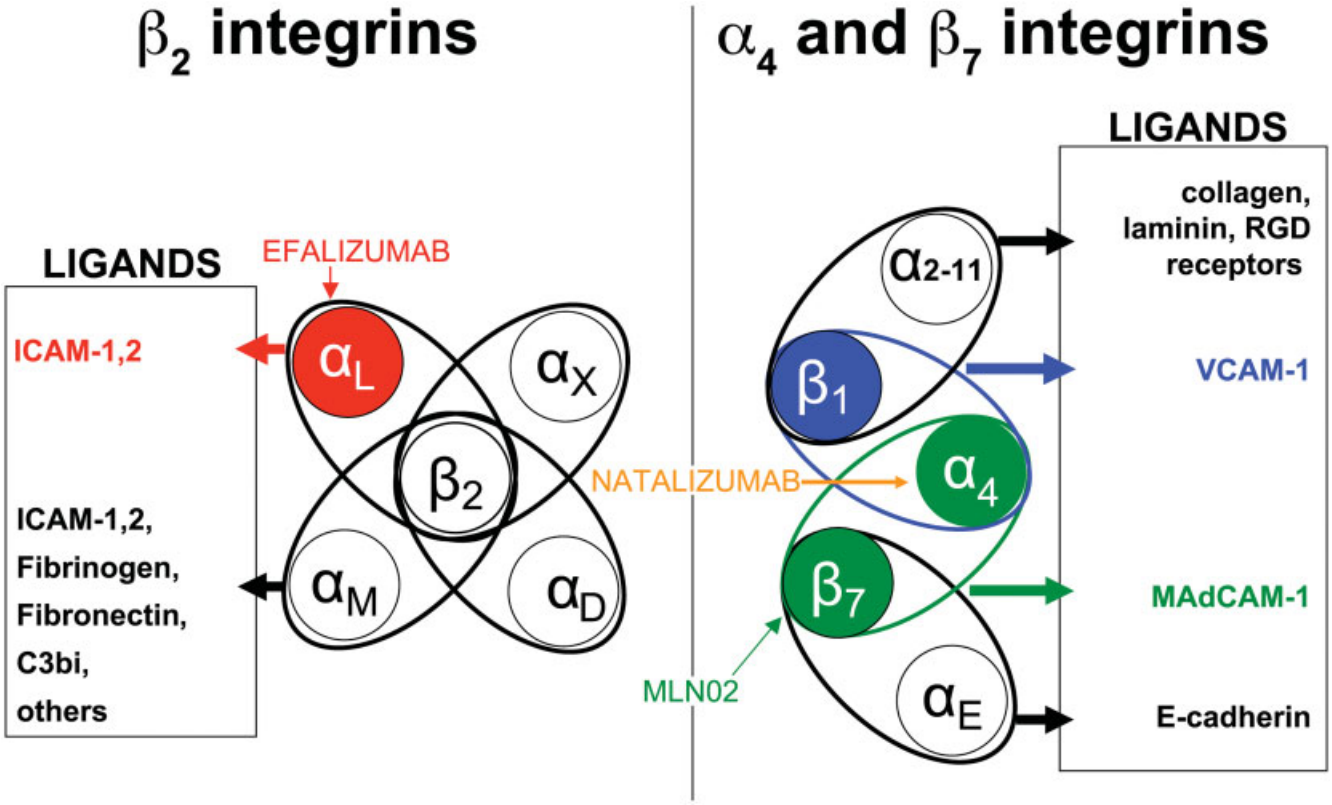

FIGURE 3. The integrins are $\alpha\beta$ heterodimers; each subunit crosses the membrane once. Mammalian integrins form subfamilies that share common subunits that bind distinct ligands. Illustrated in color are the integrins that have been targeted for the treatment of human diseases. RGD: arginine-glycine-aspartic acid sequence found in some integrin ligands. C3bi: Complement 3b inactivated.

mosiamose), developed by Texas Biotechnology (www.tbc-.com), has been tested as an inhaled formulation for pediatric asthma and in psoriasis.[138] Positive results were reported from a Phase II clinical trial in patients with chronic obstructive pulmonary disease (COPD (http://www.revotar.de/news_and_press.php).

Integrins

Integrins are a large superfamily of heterodimeric transmembrane glycoproteins that allow attachment of cells to extracellular matrix proteins or to ligands on other cells. Integrins contain large (α) and smaller (β) subunits of 120–170 kDa and 90–100 kDa, respectively. Integrins contain binding sites for the divalent cations Mg^{2+} and Ca^{2+}, which are necessary for their adhesive function. Distinct families of mammalian integrins form through the association of specific α subunits with different β subunits[25] (Fig. 3). The families of greatest importance for leukocyte adhesion in inflammation and immunity are the β_2, α_4, and β_7 families of adhesion molecules. Unlike the selectins, they establish stable protein–protein interactions and mediate arrest of cells on the vessel wall. However, some integrins have also been shown to participate in rolling.[139] Integrins are also important signaling molecules involved in many cellular processes.[140] In addition, several β_1 integrins are involved in lymphocyte interaction with ECM proteins.[25]

β_2 Integrins (CD18)

Integrins containing the β_2 subunit (CD18) are exclusively expressed on bone marrow-derived cells. β_2 integrins undergo a conformational change upon cellular activation (inside-out signaling), which is necessary for ligand binding.[140,141] Activation is initiated by the binding of a chemokine or other chemoattractant to its heptahelical G-protein coupled receptor (GPCR) on the leukocyte surface.[41,142] Ligation of the GPCR results in integrin activation within less than a second.[140]

β_2 integrins include 4 different heterodimers: CD11a/CD18 (LFA-1) is the predominant β_2 integrin on lymphocytes and neutrophils; CD11b/CD18 (Mac-1) is expressed by monocytes, granulocytes, and some NK cells; CD11c/CD18 (p150, p95) is present on monocytes and dendritic cells. CD11d/CD18 is expressed primarily by myelomonocytic cell lines and is predominantly found on macrophages and granulocytes in the red pulp of the spleen.[143] In humans[144] and mice,[145] null or hypomorphic mutations in the *Itgb2* gene

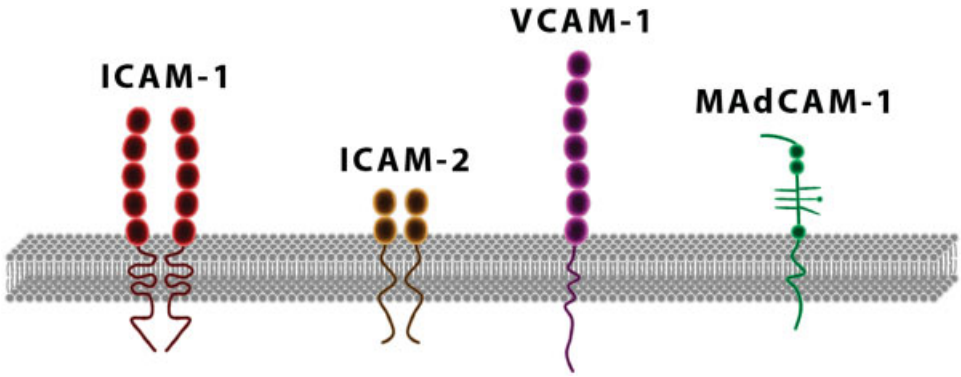

FIGURE 4. Immunoglobulin superfamily. Members of this family contain 2 to 7 immunoglobulin domains and serve as ligands for leukocyte integrins. ICAM-1 and ICAM-2 exist as homodimers on the cell surface.

encoding the β_2 (CD18) integrin subunit result in the genetic disorder LAD-1.[144] Patients with LAD-1 have recurrent bacterial infections due to ineffective recruitment of granulocytes in response to infections.[144] This spontaneous human disease dramatically illustrates the important role played by CD18 integrins in innate immunity.

LFA-1 binds to intercellular adhesion molecule-1 (ICAM-1) and ICAM-2 on the endothelium.[146,147] Its crucial role has been demonstrated in many cellular processes, such as migration, antigen presentation, and cell proliferation. Mice homozygous for a null mutation in the gene encoding the LFA-1 α chain have, among other defects, a mild defect in lymphocyte homing to secondary lymphoid organs.[148] Targeted blockade of the integrin αL subunit (CD11a) of LFA-1 (i.e., efalizumab) has been approved by the FDA for the treatment of chronic plaque psoriasis.[7] LFA-1 shares several characteristics and functions with Mac-1. LFA-1 has a predominant role for adhesion, Mac-1 is necessary for respiratory burst and certain forms of phagocytosis.[149,150] Mac-1 has no known function on lymphocytes. The other β_2 integrins, i.e., $\alpha_x\beta_2$ (CD11c/CD18) and $\alpha_d\beta_2$ (CD11d/CD18) are not expressed on lymphocytes.

α_4 Integrins (CD49d)

An important member of the α_4 family of integrins for leukocyte adhesion and trafficking is very late antigen-4 (VLA-4, CD49d/CD29, $\alpha_4\beta_1$).[151,152] VLA-4 binds to its ligand VCAM-1, and is chiefly responsible for lymphocyte and monocyte adhesion to vascular endothelium. Studies in autoimmune encephalitis (EAE) have demonstrated a critical role for VLA-4 for lymphocyte homing into the CNS. VCAM-1, which is expressed at very low levels in CNS microvessels, is highly induced by proinflammatory stimuli in EAE and in patients with MS.[153]

$\alpha_4\beta_7$ is a pivotal integrin involved in gut-homing. This integrin binds to MAdCAM-1[129,130] and plays a critical role in homing of lymphocytes to Peyer's patches by supporting their binding to HEVs. Both $\alpha_4\beta_1$ and $\alpha_4\beta_7$ can be activated to a high-avidity state.[154] The β_7 integrin null mouse shows reduced homing of naïve lymphocytes to Peyer's patches,[48] which is even further reduced in double mutant mice lacking both β_7 integrin and L-selectin.[31,155] Homozygous null mutations for integrins α_4 or β_1 lead to embryonic lethality.[156] α_4-null chimeric mice have normal thymus cellularity and circulating T cells at birth, yet after a few weeks the thymus becomes atrophic.[157,158] These data support an important role for α_4 integrins for the maintenance of thymocyte populations after birth. Integrin β_7 deficiency does not have a comparable effect.

Integrin $\alpha_E\beta_7$ (CD103)

Integrin $\alpha_E\beta_7$ (CD103) was first identified by a monoclonal antibody that recognizes lymphocytes in intestinal tissue (i.e., human mucosal lymphocyte antigen-1, HML-1). The 175-kDa alpha E chain pairs exclusively with the β_7 chain also present in $\alpha_4\beta_7$. Integrin $\alpha_E\beta_7$ is expressed by over 90% of intraepithelial lymphocytes and subsets of dendritic cells (DCs). Its role in lymphocyte traffic remains unclear; however, recently it has emerged as an important marker of $CD4^+$ and $CD8^+$ regulatory T cells as well as for DC subsets responsible for imprinting gut tropism to T cells.[159–161]

Immunoglobulins

Members of the immunoglobulin superfamily share structural and genetic features with immunoglobulin molecules, and each contains at least 1 immunoglobulin domain (Fig. 4). An immunoglobulin domain consists of two β-pleated sheets held together by a disulfide bond. Some molecules of the immunoglobulin superfamily are expressed on the vascular endothelium, where they act as counter-receptors for leukocyte integrins. Three of these immunoglobulins are of particular importance for leukocyte adhesion in IBD. ICAM-1 or CD54, VCAM-1 or CD106, and MAdCAM-1 all play important roles in the dysregulated trafficking of leukocytes that occurs during IBD.

ICAM-1

ICAM-1 is a homodimeric molecule constitutively expressed on a variety of cell types including resting endothelial cells. Its expression on endothelial cells is upregulated by stimulation with cytokines.[162] ICAM-1 is one of the principal ligands for the β_2-integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18),[163] although in the context of transmigration it seems that CD11a predominantly binds to ICAM-1, whereas CD11b binds to multiple ligands.[164] Under resting conditions the affinities of LFA-1 and Mac-1 for ICAM-1 are low,[139] but activation of leukocytes causes conformational changes in LFA-1 and Mac-1 that greatly increases their affinities for ICAM-1. Although ICAM-1 cannot support rolling on its own, isolated LFA-1 I domain supports rolling on ICAM-1.[165] On neutrophils, E-selectin engagement can induce partially activated LFA-1 to mediate LFA-1-dependent rolling on ICAM-1.[72] An antisense oligonucleotide that blocks the translation of ICAM-1 mRNA (i.e., Alicaforsen) has been evaluated in IBD, yet its efficacy has been limited.[166]

ICAM-2

ICAM-2, like ICAM-1 is a homodimeric molecule.[147] Different from ICAM-1 which has 5 Ig-like domains, ICAM-2 has only 2 (Fig. 4). It is expressed constitutively on endothelial cells, including HEV, on certain leukocytes and on megakaryocytes and platelets.[167] The expression of ICAM-2, different from ICAM-1 does not appear to be upregulated by inflammatory stimuli, thus it is thought to be involved mostly during constitutive trafficking. ICAM-2-deficient mice have normal leukocyte homing and maturation and do not suffer from spontaneous inflammatory conditions.[168]

VCAM-1

VCAM-1 (CD106) is a type-1 transmembrane protein that contains either 6 or 7 immunoglobulin domains[169,170] and is expressed on both large and small vessels after stimulation by cytokines.[171–173] Expression on resting endothelial cells is very low or absent. On cultured endothelial cells, sustained expression of VCAM-1 lasts at least 24 hours. VCAM-1 may also be found on epithelial cells, dendritic cells, Kupffer cells, and on smooth muscle cells within atherosclerotic lesions.[173–175] VCAM-1 is primarily an endothelial ligand for very late antigen-4 (VLA-4 or $\alpha_4\beta_1$) and promotes the adhesion of lymphocytes, monocytes, eosinophils, and basophils. VCAM-1-deficiency results in embryonic lethality. A conditional VCAM-1-mutant mouse that lacks VCAM-1 on endothelial cells showed a lymphocyte homing defect to bone marrow and impaired humoral responses.[176,177] Mice hypomorphic for VCAM-1 expression were protected from atherosclerosis.[178]

MAdCAM-1

MAdCAM-1 contains 2 immunoglobulin domains and a mucin domain,[129] and is the main ligand for $\alpha_4\beta_7$ integrin.[130] It is expressed at high levels by HEV of Peyer's patches and in lamina propria venules.[129] The frequency of MAdCAM-1-positive vessels is increased in human IBD[179] and in animal models of colitis.[180] MAdCAM-1 is also present in the inflamed pancreas[181] and may be inducible in other locations by proinflammatory cytokines such as TNF-α and IL-1.[182]

In addition to binding to $\alpha_4\beta_7$ integrin, MAdCAM-1 isolated from young mice also supports rolling through L-selectin.[131] This double role of MAdCAM-1 may underlie the synergistic effects of L-selectin and $\alpha_4\beta_7$ integrin in lymphocyte homing to gut-associated lymphoid tissue.[31,44] MAdCAM-1 is upregulated in the IL-10 knockout mouse, which develops spontaneous colitis,[180] and in intestinal lesions of patients with IBD,[179] supporting an important role in chronic inflammatory conditions.

ANIMAL MODELS OF COLITIS

As there are excellent reviews on the topic,[183] we will only briefly introduce models in which the anti-adhesion strategy has been evaluated with the goal of attenuating inflammation. Three broad categories of colitic models have been used to explore this strategy. A spontaneous model (i.e., the cotton-top tamarin model), 4 chemically induced models (i.e., acetic acid-, 2,4,6-trinitrobenzenesulfonic acid [TNBS], dextran sulfate sodium [DSS], and formalin-induced colitic models) and 2 others that develop colitis due to deficient regulatory responses (i.e., CD45Rb^{high} CD4 lymphocyte transfer, IL10-deficient mice).

Cotton-top Tamarins

A wasting syndrome was reported in cotton-top tamarins (CTTs), an endangered South American marmoset, in 1976. An association between colitis and colonic adenocarcinoma in CTT was reported in 1981[184] and their chronic colitis formally characterized by Madara et al in 1985.[185] Similar to patients with UC, CTT develop recurrent spontaneous flares of colitis with distortion of the mucosal architecture, suggesting chronic dysregulated immune responses. No identifiable pathogens to which the histologic findings may be attributed were identified. Like human IBD, colitis in CTT responds to treatment with the 5-ASA compound sulfasalazine.[185]

Chemically Induced Models

The administration of diverse chemical agents such as sulfated polysaccharides (e.g., carrageenan, amylopectin sulfate, DSS), rectal instillation of diluted acetic acid, TNBS, and intravenous injection of immune complexes followed by chemical irritation of the colon all result in the development

of colitis. A common shared mechanism for all of these models is the disruption of the epithelial barrier with increased exposure of intestinal microflora to the intestinal immune system. The observation that administration of such distinct chemical irritants result in similar histologic findings suggests that the colitis is rather an unspecific, stereotypic response to injury. The original disturbance does not determine the specific nature of the resulting lesions but likely serves as a trigger of common immunologic responses. The inflammatory response in all of these models is self-limited and resolves spontaneously upon removal of the injurious agent. Therefore, these models do not accurately replicate the specific chronic pathogenetic mechanisms that mediate disease maintenance in human UC or colonic CD.

Acetic Acid

Intrarectal administration of acetic acid (AA) results in barrier dysfunction and induces diffuse colitis in a dose-dependent manner. At the tissue level, AA induces diffuse ulcers of the distal colon with alterations in crypt architecture with occasional transmural inflammatory infiltrates that vary according to the severity of the process.[186] As an acute colitis, it is better suited to address the early pathogenetic mechanisms of IBD lesions.

2,4,6-Trinitrobenzenesulfonic Acid (TNBS)

Intracolonic coadministration of 50% ethanol, which breaches the mucosal barrier, along with the hapten TNBS induces a sustained colitis that persists up to 8 weeks. The inflammatory infiltrate is mixed, composed of neutrophils, macrophages, and lymphocytes. Granulomas are present in over 50% of animals. Disruption of the barrier by ethanol and TNBS are both required for the induction of colitis and administration of one or the other fails to induce disease.[187] As the inflammatory process lasts for up to 2 months, additional information may be obtained beyond the very early developmental stages of colitis.

DSS

DSS administered orally is a reproducible and technically simple way to induce colitis in rodents. Differential susceptibility to DSS has been reported in several mouse strains, yet the agent induces some degree of colitis in all mice. DSS disrupts the mucosal barrier and induces a colitic process that is more dependent on innate immune responses, as it develops even in the absence of T, B, and NK cells.[188] Yet in mice with an intact immune system there is eventual activation of T-cell responses, which is superimposed on the primary innate responses induced by the chemical agent.

Formalin-induced

This model is part of an early family of IBD animal models induced by deposition of immune-complexes in the colon, triggered by the mucosal injury that results after administration of dilute formalin. The lesion is mostly localized to the mucosa with damage to the epithelium, loss of the goblet cells, and a predominant acute inflammatory infiltrate.[189] Although some of these features are shared with human IBD, the resultant inflammatory process is transient and resolves spontaneously.

Colitic Models Due to Deficient Regulatory Responses

$CD45Rb^{high}$ Transfer Model

Powrie et al[190] described in 1994 that transfer of $CD45RB^{high}$ cells into immunodeficient recombinase activating gene (RAG)-deficient or severe combined immunodeficient (SCID) mice results in the development of colitis. Most naïve T cells are $CD45RB^{high}$, whereas regulatory T cells are not within this subset. Cotransfer of the $CD45RB^{low}$ cells, which contain regulatory T-cell subsets, prevents and cures colitis. The bacterial flora remains an important factor in this model, as transfer of $CD45RB^{high}$ cells into gnotobiotic recipients results in attenuated disease.[191] Different from previously discussed models, adaptive immune responses are fundamental in $CD45RB^{high}$ colitis, enabling the assessment of the role of adhesion molecules expressed by pathogenic T cells and the contribution of specific T-cell subsets to the disease process.

IL10-deficient Mouse

IL-10-deficient mice develop chronic transmural colitis.[192] The disease is characterized by T-helper (Th)1 responses during early stages and by Th2 responses during late stages. Lymphocyte development and antibody responses are normal. Histological findings in the colon include mucosal hyperplasia, mixed inflammatory infiltrates, and aberrant expression of major histocompatibility complex class II molecules on epithelia. The bacterial flora remains an important player, as mice kept under specific pathogen-free conditions develop only local inflammation within the proximal colon. Thus, IBD in this model results from dysregulated immune responses likely triggered by bacterial antigens. IL-10 is probably an essential regulatory cytokine for the maintenance of intestinal homeostasis. The inflammatory process is chronic and therefore suited to address mechanisms related to the chronic stages of IBD.

Adhesion Molecules as Therapeutic Targets in Animal Models of Colitis

Interference with lymphocyte recirculation to the intestine by targeting specific adhesion molecules and chemokine receptors is an attractive strategy for drug development in IBD.[19] This approach has already resulted in the development of several agents that have reached clinical use, e.g., natalizumab (Biogen/Elan Pharmaceuticals, www.tysabri.com), which targets the α_4 integrin, shared by the $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins. MLN02 (Millennium Pharmaceuticals, www.

TABLE 1. Adhesion Molecule Blockade in Animal Models of IBD

IBD Model	Adhesion Molecules	Effect	Reference
CTT	E-selectin	No attenuation in colitis	193
TNBS	E-, L- and P-selectin	Decrease in rolling flux; no attenuation in colitis	195
DSS	P-selectin	Attenuation in the severity of colitis	196
	PSGL-1	Reduction in number of rolling leukocyte; increased rolling velocity; attenuated colitis	199
SAMP/YitFc	PSGL-1	Attenuation of transfer and spontaneous ileitis	193
	E-, L- and P-selectin	No attenuation in ileitis	
TNBS	β2-integrin	Decreased neutrophil infiltration and Epithelial injury; attenuation in the severity of colitis	201
	αMβ2-integrin	Attenuation of colitis	202
TNBS-/CD45RB^{high}	αMβ2 and αXβ2 integrin	Amelioration of colitis	206
CTT	α4 integrin	Attenuated acute but not chronic inflammatory infiltrates	193
	α4β7 integrin	Attenuation of colitis	207
Gαi2	α4 integrin	Exacerbation of colitis in long term treatment	246
CD45RB^{high}	α4β7 integrin/MAdCAM-1	Blockade of lymphocyte recruitment; reduction in severity of colitis	208
DSS	MAdCAM-1	Decreased number of β7 integrin positive cells; reduction in colonic injury	209
SAMP/Yit	MAdCAM-1	Amelioration of ileitis; attenuation of established ileitis	247
SAMP/YitFc	α4β7 integrin/MAdCAM-1	No effect on the severity of acute and chronic ileitis	90

mlnm.com/rd/inflammation/candidates/mln02.asp) targets specifically the $\alpha_4\beta_7$ integrin. Most recently, Traficet EN (ChemoCentryx, Mountain View, CA), which targets the small-intestinal-specific chemokine receptor CCR9, has been tested in IBD. The therapeutic success of natalizumab has been overshadowed by the appearance of serious complications.[21] Numerous studies have targeted adhesion molecules in animal models of IBD (Table 1), yet much more remains to be learned. A greater knowledge of the basic mechanisms that underlie dysregulated chronic inflammatory trafficking, as well as the differences between trafficking to the small or large intestine, will be required for the further development of the anti-adhesion strategy in IBD.

Targeting Selectins in Murine Models of Colitis

Given the critical role of the selectins in mediating the initial steps of leukocyte recruitment to inflammatory sites, they would appear to be obvious potential therapeutic targets in IBD. Yet few reports attempted to target these molecules in animal models of IBD or reported disease attenuation by targeting a selectin. In the earliest report by Podolsky et al.,[193] colitic Cotton-top tamarins, which suffer from a spontaneous relapsing colitis reminiscent of UC, were administered 2 different antibodies against E-selectin. Despite close attention to dosing, half-life, and tissue levels, these antibodies had no significant attenuating effect on the severity of the colitis. In a subsequent report, colitis was induced in P-selectin-deficient, and P- and E-selectin double-deficient mice using acetic acid or TNBS. Damage scores were not different in the P-selectin-deficient mice when compared to wildtype mice, whereas the double-deficient mice showed enhanced leukocyte recruitment and more severe disease.[194] More recently, Sans et al[195] reported a series of experiments that explored the role of the selectins in the TNBS-induced rat model of colitis. Antibodies against E-, L-, and P-selectin decreased rolling fluxes at certain timepoints, while the anti-P-selectin monoclonal antibody also decreased the number of adherent leukocytes. Yet no attenuating effect on the severity of the colitis was observed, even with the anti-P-selectin antibody. By contrast, the following year the same group assessed the effect of an anti-P-selectin antibody and P-selectin-deficiency in DSS-induced colitis in mice, and observed an attenuating effect of the severity of the colitis for both.[196] These authors propose that this discrepancy may result from interspecies variability, different experimental approaches, or distinct pathophysiological mechanisms related to the different ways of inducing colitis in the different models. In agreement with this study, anti-P-selectin antibodies interfered with leukocyte rolling and adhesion in DSS-treated mice[197] and attenuated TNBS-colitis in rats.[198]

Selectin Ligands in Colitis

We are aware of only one study that targeted selectin ligands in a mouse model of colitis. Rijcken et al[199] treated mice with an antibody against PSGL-1 (2PH1) and studied its effects on rolling and adhesion through intravital microscopy. In addition, they assessed its effect on colonic inflammation by means of myeloperoxidase assays, a surrogate marker for neutropil infiltration and histological assessment of the severity of colitis. Blockade of PSGL-1 with 2PH1 reduced the number of rolling leukocytes, increased rolling velocities, and attenuated colitis, supporting an important role for PSGL-1 for leukocyte recruitment in this model. Recently, PSGL-1 deficient $CD4^+$ T cells were shown to induce colitis in $RAG^{-/-}$ mice indistinguishable from that of wildtype cells,[200] whereas the expression of PSGL-1 in colonic endothelium was induced by DSS administration.[94] The functional role of PSGL-1 in colitis remains unclear.

Targeting Integrins in Murine Models of Colitis

β_2 Integrins in Colitis

The first report of an attenuating effect by targeting an integrin in an animal model of IBD appeared in 1992 when Wallace et al[201] administered an anti-CD18 mAb to rabbits before and after induction of colitis by TNBS. Both neutrophilic infiltration and epithelial injury improved with this strategy, regardless of whether the antibody was administered prior to or 12 hours after TNBS administration. This result was confirmed in TNBS colitis in rats using 2 antibodies against CD11b/CD18, administered 2 hours prior to and 3 days after induction of colitis.[202] Immune complex colitis induced by formalin administration in rabbits also responded to an antagonist of CD11b/CD18.[203] Recently, Grisham and colleagues[204,205] reported attenuated colitis induced by $CD4^+$ T cells isolated from LFA-1 (CD11a/CD18)-deficient ($Itgal^{-/-}$) mice, and attenuated colitis when DSS was administered to CD18 ($Itgb2^{-/-}$) and CD11a null mice. An antibody against complement receptor 3, which serves as a ligand for $\alpha_M\beta_2$ (Mac-1) and $\alpha_x\beta_2$ integrins ameliorated TNBS- and $CD45RB^{high}$-induced colitides.[206]

α_4 Integrins in Colitis

Targeted blockade of the α_4 integrins in an animal model of IBD was first described by Podolsky et al[193] in cotton-top tamarins. An anti-human α_4 integrin (HP1/2) known to interfere with the interaction of VLA-4 with VCAM-1 or a saline placebo control were administered daily for 7 days to colitic animals. Colonic biopsies obtained before and after treatment demonstrated that HP1/2 attenuated acute activity but had no effect on the chronic inflammatory infiltrates. An antibody that targeted a combinatorial epitope on the $\alpha_4\beta_7$ integrin was then successfully used to attenuate colitis in cotton-top tamarins. Following a similar protocol to that of Podolsky et al, cotton-top tamarins were randomized to receive daily injections for 7 days of anti-$\alpha_4\beta_7$ or an isotype control, with acquisition of colonic biopsies at 3 timepoints and close monitoring of a clinical effect on stool consistency. Significant histological improvement was observed. Clinically, cotton-top tamarins showed improvement in stool consistency during the first 24 hours after administration of the antibody. This therapeutic response persisted for over 2 weeks after completion of the therapeutic regimen.[207] Monoclonal antibodies against $\alpha_4\beta_7$ integrin and MAdCAM-1 blocked lymphocyte recruitment and reduced the severity of colonic inflammation in the $CD45Rb^{high}$ transfer model of colitis, 1 of the best-studied IBD murine models.[208] Similarly, anti-MAdCAM-1 antibodies significantly reduced colonic injury and decreased the number of β_7 integrin-positive cells in the colonic mucosa in mice with DSS-induced colitis.[209,210]

Targeting Immunoglobulins in Murine Models of Colitis

ICAM-1 and VCAM-1 mRNA levels are increased in inflamed intestine of both TNBS-colitic mice[195,211] and IL-10-deficient mice[212]; in the latter, mRNA levels for MAdCAM-1 were also increased. Similarly, in the $CD45Rb^{high}$ colitic model, mRNA levels for the 3 immunoglobulins increased.[213] MAdCAM-1 is upregulated in chronically inflamed small and large intestine of patients with UC and CD.[179,181] Antibodies against MAdCAM-1 ameliorate DSS-induced colitis.[214]

In TNBS-induced colitis, ICAM-1 was slightly upregulated and VCAM-1 was upregulated at least 8-fold, as measured by an intravenous radiolabeled antibody strategy useful to assess adhesion molecule expression on the endothelial surface.[215] Intravital microscopy of colonic venules revealed increased leukocyte rolling and adhesion. An antibody to VCAM-1 was able to relieve some of the symptoms of disease and also reduced leukocyte adhesion. This is probably the strongest mechanistic evidence suggesting an involvement of VCAM-1 in intestinal inflammation, although TNBS-induced colitis differs significantly from human IBD. An anti-ICAM-1 antibody also attenuated TNBS colitis in rats.[198] Colonization by *Lactobacillus casei* attenuated leukocyte recruitment to the colon, potentially by preventing the upregulation of ICAM-1 induced by TNBS.[216] Evidence for increased adhesion molecule expression in humans comes from a study based on cultured human intestinal endothelial cells isolated from IBD patients, which support increased adhesion of monocytic cells (U937) and neutrophils compared with controls,[217,218] although a molecular mechanism was not identified. In therapeutic studies, antibodies to ICAM-1 protected rats from acute colitis induced by acetic acid and DSS.[219–221] In addition, an ICAM-1 antisense oligonucleotide both prevented and reversed DSS colitis in mice[222] and ICAM-1-deficient mice showed decreased mor-

tality and attenuated colitis induced by DSS.[223] The endothelial molecule appears to be more important than leukocyte ICAM-1, as $CD4^+$ T cells isolated from ICAM-1-deficient mice induced colitis in *Rag1*$^{-/-}$ or *Rag2*$^{-/-}$ mice indistinctly from that induced by wildtype mice.[200]

ANIMAL MODELS OF CHRONIC ILEITIS

Although there is a body of literature describing the physiology of lymphocyte trafficking, our knowledge of leukocyte recruitment during conditions of chronic inflammation as seen in patients with IBD is limited. This is especially true in inflammatory cell trafficking to the small intestine, in part due to the lack of animal models that develop disease in the terminal ileum. However, two murine models that develop chronic ileitis similar to that characteristic of CD have now been described, i.e., SAMP1/Yit and TNFΔARE mice.[224,225] This has facilitated investigation of the adhesion pathways that are involved in lymphocyte trafficking specifically to the small intestine under conditions of chronic inflammation. Both the SAMP1/YitFc and TNFΔARE models of chronic ileitis show many of the features that are characteristic of CD,[183,226] including the chronic and unrelenting course, the critical role of T lymphocytes and the specific localization of the inflammatory process to the small intestine.[227]

SAMP1/Yit Model of Chronic Ileitis

In 1998, Matsumoto et al[225] described a mouse strain that develops chronic inflammation predominantly in their terminal ilea and truly recapitulated many of the histopathological findings of CD in humans. Unlike all the previously described models of IBD, intestinal inflammation develops spontaneously in the small intestine, in the absence of chemical, immunologic, or genetic manipulation. These mice were derived from the SAMP1 strain of senescent-accelerated mice (SAM), which originated from AKR inbred mice.[228] SAMP1/Yit mice represent a sub-line of the SAMP1 strain, uniquely identified by the development of spontaneous skin lesions that correlate with intestinal inflammation.[225] After selective inbreeding for the presence of skin lesions, followed by transfer from conventional to specific pathogen-free conditions, the early senescence phenotype was lost and the presence of discontinuous chronic ileitis was first described.[225]

After over 30 generations of brother–sister matings at the University of Virginia, and the appearance of several phenotypic differences not present in the donor colony, a substrain was designated SAMP1/YitFc.[229,230] Similar to the original strain, these mice have 100% disease penetrance and exhibit patchy, transmural inflammation that is primarily localized to the terminal ileum, with formation of granulomata, as well as dense infiltration of the terminal ileal lamina propria by lymphocytes, neutrophils, and macrophages. In addition, these mice develop extraintestinal manifestations that target many of the organs also affected by human IBD. These include pyoderma-like skin lesions, ocular involvement, and perianal disease, which is similar to the perianal manifestations observed in humans with CD, including fissures, rectal prolapse, fistulae, and abscess formation.[230]

Immunologically, the ileitis is characterized by increased production of IFN-γ and TNFα from lymphocytes by 4 weeks, predating any histological evidence of inflammation.[229,230] The role of Th17 cells in the pathogenesis of the disease remains to be tested. The disease is mediated by lymphocytes that infiltrate the lamina propria and mesenteric lymph nodes, display an activated phenotype, and adoptively transfer disease to SCID mice.[229] The adoptive transfer of SAMP1/YitFc $CD4^+$ T cells predominantly induces ileitis, different from that transferred by $CD45Rb^{high}$ T cells that cause colitis, which is thought to reflect the inherent ability of SAMP1/YitFc T cells to recirculate preferentially to the small intestine. The disease in these mice also responds to therapeutics currently used in the treatment of CD, including corticosteroids and monoclonal antibodies against TNFα[231] and α4 integrins, all of which ameliorate disease severity.[232]

TNFΔARE Model

A second model of chronic ileitis was described by Kontoyiannis et al in 1998.[224] The TNFΔARE model develops both a predominantly Crohn's-like chronic ileitis similar to that of SAMP/Yit mice, and deforming rheumatoid arthritis-like joint disease in both heterozygous and homozygous mice. Like SAMP1/Yit, the disease in TNFΔARE mice responds to immunoblockade of TNF-α, supporting the relevance of this model to the human diseases. TNFΔARE mice were developed by targeted deletion of 69 basepairs of AU-rich elements in the 3′ UTR region of the gene that encodes TNF-α. This deletion results in increased stability of the mRNA for TNF-α, increased synthesis of TNF-α protein, and elevated levels of systemic TNF-α.[224] Interestingly, the increased levels of TNF-α do not result in uniform pan-enteritis, but predominantly in ileitis. Homozygous $TNF\Delta ARE^{+/+}$ mice develop severe ileitis and arthritis and die at an early age. Heterozygous mice ($^{+/-}$) develop disease by 8 weeks of age that worsens progressively, yet they reach sexual maturity and procreate. A related strain (i.e., B6.129S-*Tnf*^{tm2Gkl}/Jarn, MGI: 3720980) on the BL6 background has been generated by over 20 generations of continuous backcrosses between $TNF^{\Delta ARE}$ mice on mixed genetic background (i.e., C57BL6 and 129S6), generated as previously described[224] to C57BL6/J mice. The C57BL6/J genetic background did not alter the localization, time course, or severity of the intestinal inflammation. The defined C57BL/6 genetic background greatly facilitates immunologic studies that would be difficult or impossible on the original strain with a mixed genetic background.

Trafficking Pathways in Models of Ileitis

Dissecting the lymphocyte trafficking pathways in chronic murine ileitis may help optimize the current therapeutics in clinical use in IBD, as well as to identify new potential therapeutic targets. To that effect we have targeted many of the adhesion molecules and chemokines using these two models of ileitis.

L-selectin in Ileitis

The role played by L-selectin in naïve T-cell trafficking has been relatively well characterized,[233] whereas the role it plays during induction or maintenance of chronic inflammation is incompletely understood.

Expression of L-selectin is often considered to correlate with antigenic inexperience and to be indicative of the naïve state. Although naïve T cells indeed express L-selectin, it is also expressed in central memory T cells[51] at other times. L-selectin is reexpressed after lymphocyte activation in in vitro systems, and subpopulations of memory and effector lymphocytes express L-selectin.[33,51,234] Therefore, L-selectin may continue to play a role in the recruitment of antigen-experienced subpopulations of lymphocytes important for the pathogenesis of chronic inflammatory diseases, where L-selectin ligands (expressed in $PNAd^+$ and $MAdCAM\text{-}1^+$ vessels) have been demonstrated within effector sites. $PNAd^+$ vessels are consistently found within inducible lymphoid aggregates or follicles (ILF), also known as tertiary lymphoid organs (TLO).[125,128] Thus, recruitment of L-selectin-expressing T cells likely occurs at these sites, and interference with these pathways may be of therapeutic value.[123,124]

Targeting Selectins in Ileitis

The models of chronic ileitis have allowed us to assess for the first time whether the trafficking pathways to the small and large intestine were shared or distinct from those to other intestinal segments under conditions of dysregulated inflammation. Systematic targeting of the different families of adhesion molecules with neutralizing antibodies against single molecules or in combination was performed in an adoptive transfer model in which $CD4^+$ T cells from SAMP1/YitFc mice are adoptively transferred into MHC-matched SCID mice. The predominant disease in the recipients is ileitis, not colitis. Single (data not shown) or combined blockade of P-, E-, and/or L-selectin failed to attenuate the ileitis in SCID mice or in SAMP1/YitFc mice with established ileitis[93] (Fig. 5). However, administration of the anti-L-selectin antibody MEL-14 prior to the development of inflammation had a protective effect, suggesting a requirement for L-selectin during the initiation stages of the disease (Rivera-Nieves, unpubl. results).

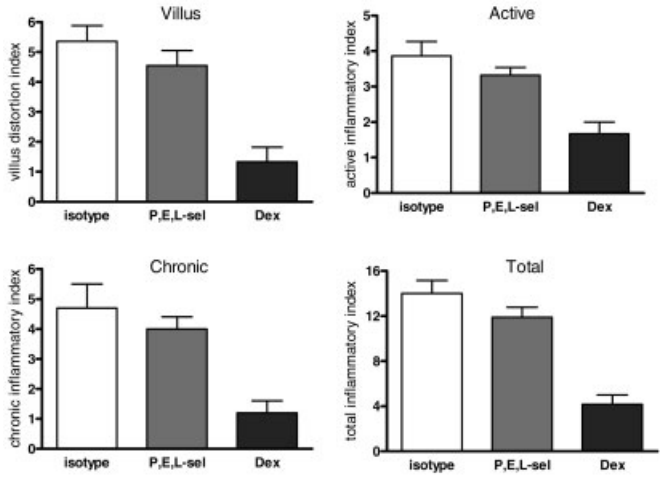

FIGURE 5. Combined selectin blockade did not significantly attenuate established ileitis in SAMP1/YitFc mice. SAMP1/YitFc mice at 10 weeks of age received antibodies against P-, E-, and L-selectins (mAb RB40.34, 9A9, MEL-14), their respective isotype antibodies combined or dexamethasone (Dex 100 μg IP) and the histological severity of the ileitis was assessed as described previously[232] (villus–villus architectural distortion, active—neutrophilic infiltrates, chronic-predominantly lymphocyte infiltrates, and total—sum of villus active and chronic indices, mean $\pm$ SEM, $n = 7$ mice/treatment group).

Targeting Selectin Ligands in Ileitis

PNAd

The selectins possess a C-type lectin domain at their amino terminus that interacts with carbohydrate determinants expressed by ligands expressed on HEVs and PSGL-1 expressed by adherent leukocytes.[26] Indeed, all of the L-selectin ligands identified to date (i.e., CD34, GlyCAM-1, sgp200, PSGL-1, endomucin, MAdCAM-1) contain carbohydrate moieties.[135] The carbohydrate posttranslational modifications found on HEVs that are necessary for L-selectin recognition include sialylation, fucosylation, and carbohydrate sulfation.[125] Chronic inflammatory processes induce the appearance of HEV-like vessels in numerous human diseases and animal models of chronic inflammation.[235] A likely role of these inflammation-induced structures is to support recruitment of L-selectin-expressing lymphocytes directly into effector sites, where during chronic inflammation numerous tertiary lymphoid organs appear.[128]

The MECA-79 monoclonal antibody stains HEVs and blocks L-selectin-dependent lymphocyte adhesion.[236] Glycoproteins carrying the PNAd epitope are recognized as major L-selectin ligands, as no additive effects on lymphocyte homing was observed after simultaneous blockade of PNAd and L-selectin. The MECA-79 epitope is found in PP, MLN, PLN but not within the intestinal LP, except in chronic inflammation.[237] Yet this pattern is not restricted to the intestine and PNAd reactivity has been reported in over 20 immune-me-

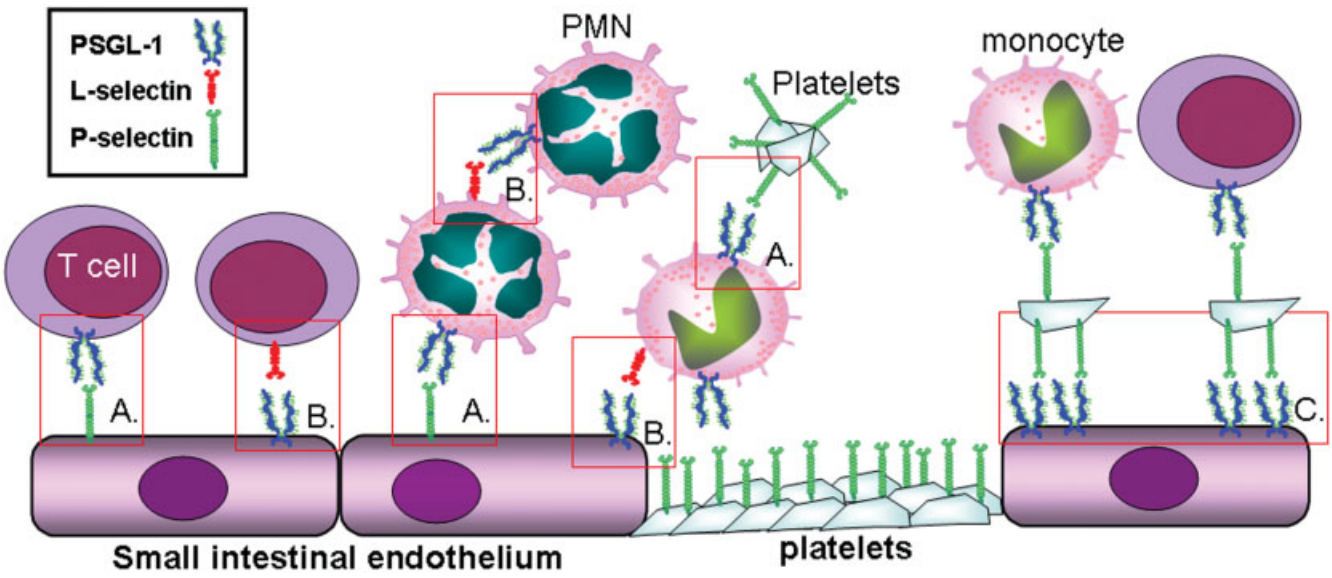

FIGURE 6. Role of PSGL-1 in leukocyte recruitment to small intestine. A: Effector T cells and other leukocytes (PMN, monocytes) express PSGL-1 which enables their interaction with P-selectin on activated endothelium or platelets. B: In addition L-selectin-expressing cells may alternatively bind to PSGL-1 on endothelium or other leukocytes. C: Platelets may also bind to endothelial PSGL-1 perpetuating dysregulated recruitment to the small intestine. Modified from Ley and Kansas, 2004.[33]

diated animal models and human conditions, including asthma, diabetes, psoriasis, RA, Hashimoto's thyroiditis, Grave's disease, cutaneous lymphoma, and allograft rejection.[125]

Immunohistochemical studies in SCID mice before and after $CD4^+$ T cell adoptive transfer from donor SAMP1/YitFc mice demonstrated that the PNAd epitope is expressed after the development of inflammation in these mice. However, single or combined blockade of MAdCAM-1 (MECA-367) and PNAd (MECA-79) provided no therapeutic benefit compared to mice treated with isotype-matched control antibodies.[93]

PSGL-1

Inoue et al[238] described for the first time the therapeutic effect of neutralization of PSGL-1 with the monoclonal antibody 2PH1 in SAMP1/Yit mice. Administration of 2PH1 to mice with established disease significantly reduced infiltration of the LP by CD4, CD8, and $CD68^+$ subsets, and the villus height was partially restored, although no effect on the hypertrophy of the muscularis (a chronic structural change) was noted. Their emphasis was on the role of PSGL-1 on monocyte recruitment to the intestine; however, when bone marrow chimeric SAMP1/Yit mice that lacked PSGL-1 in their hematopoietic compartment were generated, their disease was equal in severity to that of PSGL-1-sufficient SAMP1/Yit mice, suggesting that PSGL-1 on monocytes was not the critical molecule. The small intestinal microvasculature expressed PSGL-1 and a therapeutic effect of blockade of PSGL-1 was demonstrated in SAMP mice using a different antibody (i.e., 4RA10).[93] It is likely that the therapeutic effect of this strategy is due to its interference with multiple recruitment pathways (Fig. 6). Interfering with PSGL-1 would block rolling on P-selectin expressing endothelial cells, rolling on platelets, platelet–leukocyte interactions, and endothelial-platelet interactions.

Integrins in Chronic Murine Ileitis

The importance of the α_4 integrins in lymphocyte trafficking is well established in the literature.[4] $\alpha_4\beta_7$ and $\alpha_4\beta_1$ integrins are crucial for the dichotomy in migration to gut or to nonintestinal sites in physiological recruitment. In this setting, $\alpha_4\beta_7$-expressing cells migrate preferentially to the gut, whereas lymphocytes expressing the $\alpha_4\beta_1$ integrin (VLA-4) preferentially traffic to nonintestinal sites.[236,239] Expression of these integrins under physiological conditions is reciprocal, and gut-homing $\alpha_4\beta_7^+$ cells tend to be $\alpha_4\beta_1^{neg}$ or low and vice versa.[236,239] Recent clinical trials with a humanized mAb to α_4 integrins in patients with CD have suggested that α_4 integrins may be relevant therapeutic targets.[18,20] Yet while targeting both α_4 integrins ($\alpha_4\beta_7$, $\alpha_4\beta_1$) through its shared α_4 moiety is effective in CD, a more specific mAb that targeted $\alpha_4\beta_7$ specifically was effective in UC but not in CD.[240] These data support divergent trafficking pathways between small and large bowels. Further understanding of potential mechanisms of induction of small intestinal specific homing have recently emerged with the discovery that retinoic acid is essential for the imprinting of T cells with a small intestinal homing phenotype.[241–243]

Expansion of the β_7 integrin-expressing $CD4^+$ T cell population and of MAdCAM-1 after T cell transfer suggests a role for these molecules in the pathogenesis of chronic ileitis. Yet neutralizing monoclonal antibodies against the β_7 integrin subunit (FIB-504) or $\alpha_4\beta_7$ integrin (DATK-32) were ineffective in reducing the severity of acute or chronic infiltrates compared to isotype antibody-treated SCID mice with chronic ileitis.[90] In agreement, a β_7-deficient SAMP1/Yit substrain recently generated develops ileitis indistinguishable from that of β_7-sufficient SAMP1/Yit mice (Gorfu, Rivera-Nieves, McDuffie, Pizarro, Cominelli, and Ley, unpubl. obs.). To attenuate ileitis, blockade of at least two integrins might be required, for example, using an anti-α_4 integrin antibody, which interferes with both $\alpha_4\beta_7$ and $\alpha_4\beta_1$ integrins.[90]

Immunoglobulins in Chronic Murine Ileitis

The expression of ICAM-1 and VCAM-1 was increased in intestinal microvessels of SAMP1/YitFc mice and the related SCID adoptive transfer model mice in the presence of inflammation, consistent with their induction under conditions of chronic inflammation. In addition, combined blockade of ICAM-1 and VCAM-1 was able to attenuate ileitis in adoptively transferred SCID mice, whereas blockade of either alone was not sufficient.[232] These data support the hypothesis that the trafficking pathways under conditions of chronic inflammation are highly redundant and resistant to therapeutic intervention. This is further supported by the complete lack of efficacy by the same combined intervention in SAMP1/Yit mice, in which the inflammatory process develops over many months with the likely acquisition of highly redundant trafficking pathways.[232]

In a separate set of experiments, neutralization of MAdCAM-1 was found to be ineffective at attenuating ileitis in SCID mice, despite clear induction of this molecule with the development of chronic inflammation. It is likely that T cells continue to home to the intestine using VCAM-1/$\alpha_4\beta_1$ and ICAM-1/LFA-1 after the MAdCAM-1/$\alpha_4\beta_7$ pathway has been neutralized. This is supported by the efficacy of the simultaneous blockade of α_4 integrins (clone PS2) and ICAM-1,[232] which targets multiple mechanisms of arrest.[90]

FUTURE DIRECTIONS

Combination Anti-adhesion Molecule Therapies

During the past decade the success of anti-TNF-α strategies has revolutionized the treatment of IBD.[244] Yet only about 70% of patients respond to this therapy, driving the continued search for other antiinflammatory strategies for the treatment of the remaining 30% of patients who do not respond and for those that respond initially but eventually fail. Therefore, alternative biological therapies that target other pathways of the chronic inflammatory cascade must be evaluated in CD.[14,244] Interference with leukocyte recirculation to the intestine by targeting specific adhesion molecules has resulted in the development of agents that have advanced into clinical use, e.g., Natalizumab (Biogen/Elan Pharmaceuticals, www.tysabri.com) and MLN02 (Millennium Pharmaceuticals, www.mlnm.com/rd/inflammation/candidates/mln02.asp), which has been evaluated in CD and UC.[240]

A pivotal role for $\alpha_4\beta_7$ integrin/MAdCAM-1 in lymphocyte trafficking to the gut has become dogma under physiological conditions,[4,236] fueling the evolution of these anti-α_4 strategies in IBD. The evidence for a similar nonredundant role in chronic gut inflammation is less clear. Antibody-blocking studies in animals and humans suggest efficacy,[193,208–210] but specificity is unclear. Despite the rapid progression from the bench to bedside of the anti-α_4 therapeutic strategy,[18,193] much remains to be learned regarding its fundamental mechanism of action. Our data in the ileitis models support the hypothesis that compensatory mechanisms allow continued trafficking after neutralization of the $\alpha_4\beta_7$ integrin/MAdCAM-1 pathway that is pivotal for physiologic and pathologic traffic to the colon. Further dissection of the adhesive pathways in chronic ileitis may help to optimize current therapies already in clinical use for CD,[18] minimize risks and potentially to further understand the mechanisms of inflammatory lymphocyte recruitment to other organs as well.

Chemokines as Therapeutic Targets

Recent studies have shown that early blockade of CCL25 using a small molecule antagonist (CCX282, Traficet-EN, ChemoCentryx) effectively prevented ileitis in TNFΔARE mice, confirming a critical role for this molecule during the induction phase of chronic ileitis (Zheng Wei, ChemoCentryx, pers. commun.). However, when we targeted this molecule in a different model of ileitis (SAMP1/Yit), the strategy was only efficacious during early disease.[245] Targeting this and other chemokines alone or in combination with anti-adhesion strategies may be promising in subsets of patients with IBD. Small molecule approaches may be particularly useful as adjunctive therapies.

CONCLUSIONS AND IMPLICATIONS FOR FUTURE ANTI-ADHESION STRATEGIES

Physiological leukocyte recruitment is a highly regulated process with a limited number of decision points along every step of the adhesion cascade.[4,23,24] Expression of specific combinations of adhesion molecules on lymphocyte subpopulations determines their capacity to reach specific tissues, where cognate endothelial ligands show restricted expression (e.g., MAdCAM-1: gut versus VCAM-1: elsewhere). This results in orderly recruitment that begins and terminates physiological inflammatory responses. Under conditions of chronic inflammation, continuous dysregulated production of proinflammatory cytokines results in inappro-

priately increased adhesion molecule expression (e.g., MAdCAM-1), as well as aberrant expression of molecules not normally expressed by specific lymphocyte subpopulations and tissues, thus increasing the chances of lymphocytes contacting appropriate endothelial ligands. Therefore, when one adhesion molecule is blocked, others compensate for its deficiency. These redundant adhesive pathways favor adhesiveness over tissue specificity, resulting in failure of termination of the inflammatory response and perpetuation of dysregulated leukocyte recruitment. Further dissection of redundant adhesive pathways in chronic ileitis may help to optimize therapies already in clinical use and potentially develop new strategies for the treatment of CD that in two-thirds of patients involves the small intestine.

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