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THERAPEUTIC TARGETS FOR CROHN'S DISEASE

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ABSTRACT

Crohn's disease, one of the most common forms of inflammatory bowel disease (IBD), involves chronic and abnormal activation of the immune system leading to tissue destruction. Although the etiology of the disease is still unclear, it is known that Crohn's disease is triggered by an inappropriate immune response as the result of a complex interaction among environmental and microbial factors and the intestinal immune system. The primary and secondary goals in treating Crohn's disease are to control active disease to achieve a state of remission and to maintain remission, respectively. Aminosalicylates, corticosteroids, immunosuppressants and biologic agents are used to help control the disease by suppressing destructive immune processes, promoting healing of intestinal tissues and relieving symptoms. Nevertheless, there is no agent that alters the natural course of the disease. Thus, the search continues for effective treatments for Crohn's disease. In recent years, investigators have concentrated on identifying novel targets for therapeutic intervention. This article presents those drug targets that are currently under active investigation for the treatment of Crohn's disease.

INTRODUCTION

Inflammatory bowel disease (IBD) is a group of chronic inflammatory disorders of the gastrointestinal (GI) tract in which tissue damage and inflammation lead to long-term, often irreversible, impairment of the structure and function of the GI tract. Crohn's disease and ulcerative colitis are the most common forms of IBD. They both involve chronic inflammation and ulceration in the intestines, the result of an abnormal immune response. IBD is considered to be caused by a genetic defect, leading to alterations in the gut epithelial barrier, which increases intestinal permeability and therefore mucosal immune system exposure to normal constituents of the GI microflora. The result is the triggering of an aberrant immune response to microorganisms.

Chronic and abnormal activation of the immune system leads to tissue destruction in both diseases, with ulcerative colitis generally limited to the rectum and colon and Crohn's disease (also known as regional ileitis) extending deeper into the intestinal wall, and sometimes affecting the entire digestive tract from the mouth to the anus. Other less common forms of IBD include microscopic (or lymphocytic) colitis, diversion colitis, fulminant colitis and toxic megacolon (1-5).

The Crohn's and Colitis Foundation of America estimates that up to one million Americans have IBD. Prevalence and incidence rates are highest in northern Europe and North America (10-200 cases per 100,000 for both ulcerative colitis and Crohn's disease). A lower incidence is reported in areas such as southern Europe, Asia and the developing world, although rates continue to increase. While the rate of ulcerative colitis appears to have stabilized, the incidence of Crohn's disease has increased by as much as 6-fold in the past 25 years (1, 6-8).

The precise etiology of IBD remains unclear. It is known that both Crohn's disease and ulcerative colitis are triggered by an inappropriate immune response that occurs in genetically susceptible individuals as the result of a complex interaction among environmental factors, microbial factors and the intestinal immune system. Under normal conditions, the intestinal immune system maintains a balance between immune tolerance to commensal bacteria and food antigens, while stimulating an immune response to abnormal pathogens. In Crohn's disease, discontinuous granulomatous and transmural inflammation throughout the gut, ileum and colon is observed. The inflammation is dominated by $CD4^+$ T cells and the production of proinflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interferon gamma and the interleukins IL-2, IL-12, IL-23 and IL-18. Luminal antigens gain access to the underlying mucosal tissue via a leaky barrier and activate a dysregulated immune system in individuals who are genetically predisposed to the disease. Microbial antigens trigger and maintain the inflammatory response through molecular pattern recognition receptors expressed on different innate and adaptive immune cells. Myeloid dendritic cells recognize commensal bacteria as pathogens and enter a maturation program that changes their functional status from tolerogenic to activating, and promotes differentiation of naïve T cells to effector T cells (Th1, Th17 and Th2). Intestinal epithelial cells also express costimulatory molecules, enabling them to function as antigen-presenting cells and thus further contributing to the effector T-cell response (2, 3, 5, 7, 9, 10).

Results from studies in twins have revealed that a family history of IBD is the greatest independent risk factor. Both Crohn's disease and

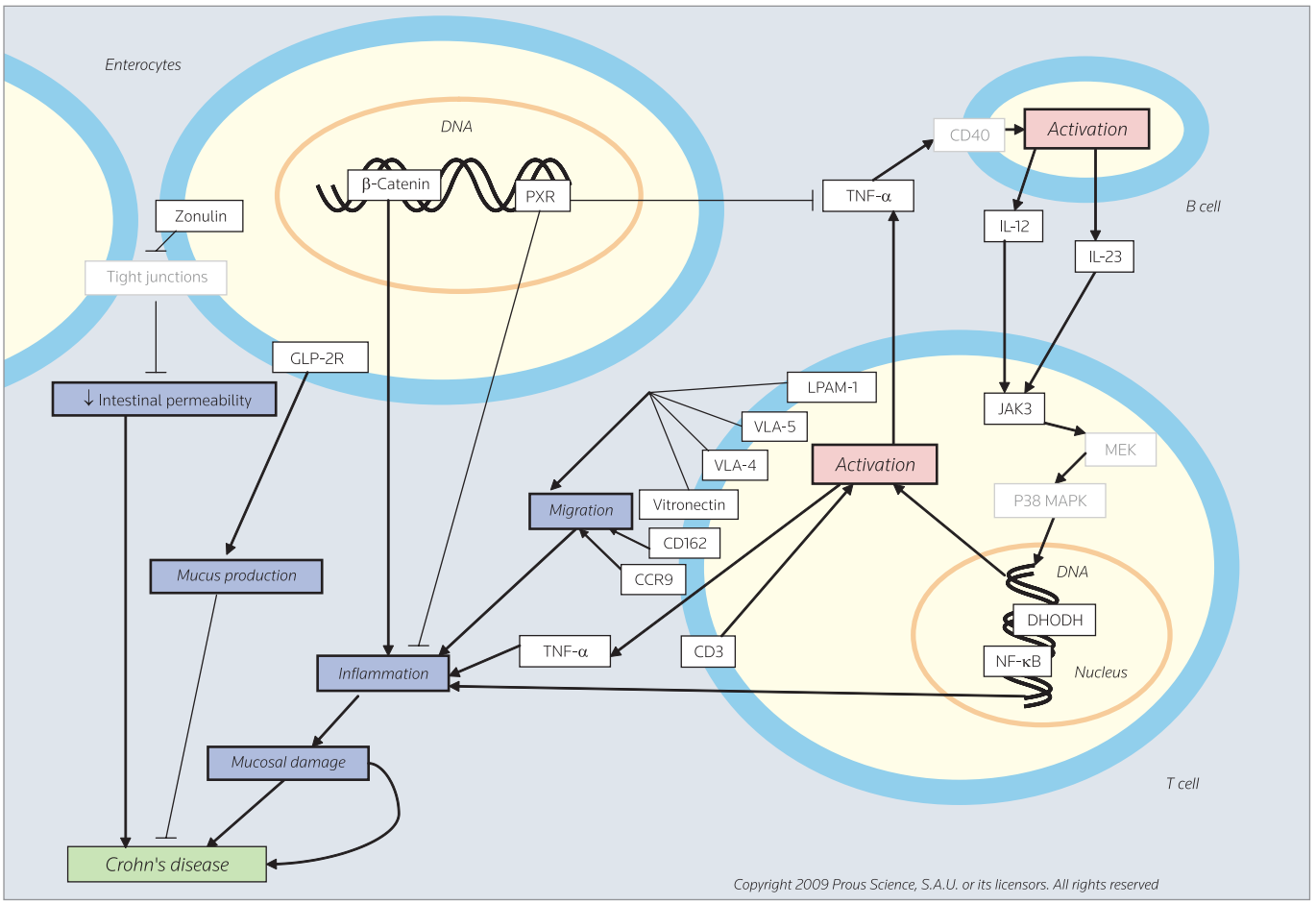

Figure 1. Crohn's disease targetscape. A diagram showing an overall cellular and molecular landscape or comprehensive network of connections among the current therapeutic targets for the treatment of Crohn's disease and their biological actions. Arrow: positive effect; dash: negative effect. Gray or lighter symbols are targets that are not validated. Abbreviations: CCR9: chemokine receptor 9; CD3: CD3 antigen (T-cell surface glycoprotein); CD40: CD40 antigen; CD162: P-selectin glycoprotein ligand 1 (PSGL-1); DHODH: dihydroorotate dehydrogenase; GLP-2R: glucagon-like peptide 2 receptor; IL-12: interleukin-12; IL-23: interleukin-23; JAK3: Janus kinase 3; LPAM-1: integrin $\alpha_4\beta_7$; MEK: dual specificity mitogen-activated protein kinase kinase; NF-κB: nuclear factor NF-kappa-B; P38 MAPK: p38 mitogen-activated protein kinase; PXR: pregnane X receptor (nuclear receptor subfamily 1 group I member 2); TNF-α: tumor necrosis factor alpha; Vitronectin: integrin $\alpha_v\beta_3$ (vitronectin receptor); VLA-4: integrin $\alpha_4\beta_1$; VLA-5: integrin $\alpha_5\beta_1$.

ulcerative colitis are polygenic diseases. Genetic variations in the *NOD2* (also known as *IBD1*) gene known to be implicated in inflammatory processes, in addition to *ATG16L1*, an autophagy gene that, like *NOD2*, affects intracellular processing of bacterial components, are thought to contribute to the development of Crohn's disease. Other genes implicated in the development of both Crohn's disease and ulcerative colitis include *STAT3, JAK2, ICOSLG, CDKAL1* and *ITLN1* (2, 7, 11).

The primary goal when treating a patient with IBD is to control active disease and achieve remission, and the secondary goal is to maintain remission. Local aminosalicylates are used to treat mild to moderate distal disease, while oral formulations are preferred in more severe disease. Corticosteroids are often administered to patients with severe disease, while fulminant attacks are controlled with intravenous ciclosporin or colectomy. However, there is no evidence that corticosteroids alter the disease course, and due to their numerous side effects, maintenance therapy with corticosteroids should be avoided. The immunosuppressant azathioprine may be beneficial in treating chronic active disease, and both azathioprine and the immunomodulator mercaptopurine heal the mucosa and are indicated for maintenance of remission in patients with moderate to severe Crohn's disease. Unfortunately, their onset of action is slow. Biologic therapy for IBD, including Crohn's disease, was initiated in 1998 with the approval of the anti-TNF monoclonal antibody infliximab, later followed by adalimumab and certolizumab pegol in 2007 and 2008, respectively. However, these anti-TNF agents have not been as effective as anticipated and the safety issues associated with them (e.g., serious infections, malignancies, neurological disease) complicate their use clinically. None of the currently available drugs provides a cure for Crohn's disease, although agents can help to control disease by suppressing destructive immune processes, promoting healing of intestinal tissues and relieving symptoms (diarrhea, abdominal pain and fever). While immunomodulators and infliximab can maintain clinical and endoscopic remission, there is little evidence that existing therapies can alter the natural course of the disease (1, 12, 13).

Table I. *Selected targets and products launched or being actively investigated for Crohn's disease (from Prous Science Integrity®).*

Target	Product	Source	Phase
β-Catenin	Mesalazine	Ferring	L-1989
CD3 antigen	NI-0401	NovImmune	I/II
Chemokine receptor CCR9 (isoform A)	CCX-282	ChemoCentryx	II/III
Dihydroorotate dehydrogenase	4SC-101	4SC	II
Glucagon-like peptide 2 (GLP-2) receptor	Teduglutide	NPS Pharmaceuticals	II
Integrin $\alpha_4\beta_1$ (VLA-4)	Natalizumab AJM-300	Biogen Idec/Elan Ajinomoto	L-2008 II
Integrin $\alpha_5\beta_1$ (VLA-5)	ATN-161	Attenuon	Preclinical
Integrin $\alpha_4\beta_7$ (LPAM-1)	Vedolizumab AJM-300	Millennium Pharmaceuticals Ajinomoto	III II
Integrin $\alpha_v\beta_3$ (vitronectin receptor)	ATN-161	Attenuon	Preclinical
Interleukin IL-12A, IL-12B	ABT-874	Abbott	II
Interleukin IL-23	ABT-874	Abbott	II
Janus kinase 3 (JAK3)	CP-690550	Pfizer	II
Nuclear factor NF-kappa-B (NF-κB)	HMPL-004	Hutchison China MediTech	II
Pregnane X receptor (PXR)	Rifaximin	Alfa Wassermann/Salix	II
P-selectin glycoprotein ligand 1 (PSGL-1, CD162)	Anti-PSGL-1	Selexys	Preclinical
TNF-α	Infliximab Adalimumab Certolizumab pegol HMPL-004 TNF-alpha kinoid LMP-420	Centocor Ortho Biotech Abbott UCB Hutchison China MediTech Neovacs LeukoMed	L-1998 L-2007 L-2008 II I/II Preclinical
Zonulin	Larazotide acetate	Alba Therapeutics	I

Despite the fact that the exact cause and mechanisms of Crohn's disease are not known, many advances have been made in recent years regarding the mechanisms underlying intestinal inflammation, which has led to the identification of new therapeutic targets and the design of novel agents to selectively shut down intestinal inflammation. The search for effective treatment strategies for Crohn's disease continues. Those targets which are currently under active investigation are discussed below (see Figure 1). Table I provides a selection of products under active development for each target and Table II includes selected patents.

TARGETS

β-Catenin

Catenins are proteins that complex with cadherin cell adhesion molecules and include α, β, γ and δ isoforms. α-Catenin binds to β-catenin and can also bind actin. β-Catenin contains armadillo repeats and is able to bind to other proteins. Within a cell, β-catenin complexes with cadherins, transcription factors and other proteins such as the scaffolding protein axin. The ability of β-catenin to bind to other proteins is regulated by tyrosine and serine kinases such as glycogen synthase kinase-3 (GSK-3). When β-catenin is not assembled in complexes with cadherins, it can form a complex with axin. While bound to axin, β-catenin can be phosphorylated by GSK-3, which creates a signal for the rapid ubiquitin-dependent degradation of β-catenin by proteosomes. Various signals, such as those generated by the Wnt signaling pathway, can inhibit GSK-3-mediated phosphorylation of β-catenin, allowing β-catenin to translocate to the cell nucleus, interact with transcription factors and regulate gene transcription. β-Catenin can be phosphorylated by other kinases, such as protein kinase A (PKA). PKA-mediated phosphorylation of β-catenin is associated with a reduction in β-catenin degradation and thus an increase in nuclear levels of β-catenin and increased interaction of β-catenin with TCF (T-cell factor) transcription factors to regulate gene expression. β-Catenin is a downstream target of the Wnt canonical pathway, which plays an essential role in cell proliferation and differentiation. Deregulated β-catenin protein levels are suspected to be involved in the pathogenesis of cancer; constitutive activation of the Wnt canonical pathway is upregulated and promotes tumorigenesis in a variety of cancer types. The E-cadherin–catenin complex (including β-catenin) is crucial for the maintenance of intestinal epithelial architecture and defects in intestinal epithelial permeability (i.e., increased permeability) are observed in patients with Crohn's disease. These changes cannot be completely explained by known IBD susceptibility genes. E-cadherin has been shown to be upregulated and disrupted in Crohn's disease and ulcerative colitis. During inflammation, structural or functional alterations in the E-cadherin–catenin complex may occur, which can result in loss of intercellular adhesion. Thus, upregulation of this complex in epithelium may occur in order to maintain its normal architecture under inflammatory conditions. The increased permeability observed in Crohn's disease could be explained by the mislocalization of E-cadherin and β-catenin and the elevation of

Table I. *Selected patents for targets being pursued or explored for Crohn's disease (from Prous Science Integrity®).*

Target	Patent	Source	Phase
Chemokine receptor CCR9	WO 2003099773	Millennium Pharmaceuticals	Biological testing
	WO 2005113513	ChemoCentryx	Biological testing
	WO 2009044311	Encysive Pharmaceuticals	Biological testing
	WO 2009017719	Encysive Pharmaceuticals	Biological testing
Glucagon-like peptide 2 (GLP-2) receptor	WO 2008056155	Zealand Pharmaceuticals	Biological testing
Integrin $\alpha_4\beta_1$ (VLA-4)	JP 2005255675	Mitsubishi Tanabe Pharma	Biological testing/Preclinical
	WO 2004103967	Genentech	Biological testing
	WO 2004014859	Mitsubishi Tanabe Pharma	Biological testing/Preclinical
	WO 2005077914	Janssen Pharmaceutica	Biological testing
	WO 2005077915	Janssen Pharmaceutica	Biological testing/Preclinical
	WO 2007101165	Elan Pharmaceuticals	Biological testing
Integrin $\alpha_4\beta_7$ (LPAM-1)	WO 2004103967	Genentech	Biological testing
	WO 2005077914	Janssen Pharmaceutica	Biological testing
	WO 2005077915	Janssen Pharmaceutica	Biological testing/Preclinical
Interleukin IL-12	WO 2005046603	Synta Pharmaceuticals	Biological testing
	WO 2005046604	Synta Pharmaceuticals	Biological testing
	WO 2006124662	Synta Pharmaceuticals	Biological testing
	WO 2007100759	Synta Pharmaceuticals	Biological testing/Phase II
	US 6384032	SBR Pharmaceuticals	Preclinical
	US 2002082259	Synta Pharmaceuticals	Preclinical
Nuclear factor NF-kappa-B (NF-κB)	WO 2006069182	Wyeth Pharmaceuticals	Biological testing
Peroxisome proliferator-activated receptor PPARα	WO 2008108735	Albireo	Biological testing
Phosphodiesterase PDE4	WO 2000014083	InflaZyme Pharmaceuticals	Biological testing
	WO 2006111549	Boehringer Ingelheim	Biological testing
TNF-α	WO 1995005849	Pharmexa	Preclinical
	WO 1998002430	Pfizer	Biological testing
	WO 2002085916	University of North Carolina, Chapel Hill/LeukoMed	Preclinical
	WO 2002094266	Procter & Gamble	Biological testing
	WO 2004039806	Ajinomoto	Biological testing
	WO 2006087644	GlaxoSmithKline	Biological testing
	US 5643893	University of North Carolina, Chapel Hill	Biological testing/Preclinical
	US 5717100	Merck & Co.	Biological testing
	US 2005113392	Procter & Gamble	Biological testing

β-catenin transcriptional activity associated with chronic inflammatory injury. Inhibition of β-catenin may therefore be a therapeutic option for the treatment of Crohn's disease (14-16).

CD3 antigen (T-cell surface glycoprotein)

The CD3 antigen is a protein complex composed of four distinct chains: CD3 gamma chain, CD3 delta chain and two CD3 epsilon chains. These chains are highly homologous cell-surface proteins that are members of the immunoglobulin (Ig) superfamily and contain a single extracellular Ig domain. The transmembrane region of these CD3 chains is negatively charged, allowing them to associate with the positively charged T-cell receptor (TCR) chains (TCR alpha and TCR beta). The intracellular tails of the CD3 chains contain a single conserved motif known as an immunoreceptor tyrosine-based activation motif (ITAM), which is essential for the signaling capacity of the TCR. Association of the CD3 chains with TCR and the zeta chain (accessory molecules of TCR) generates an activation signal in T lymphocytes. Thus, the TCR complex is composed of the TCR zeta chain and CD3 molecules. $CD3^+$ T cells are increased in patients with Crohn's disease. Therefore, modulation of the CD3 complex on T cells may be beneficial in the treatment of the disease (17, 18).

CD40 antigen

CD40 is a member of the TNF receptor superfamily that is expressed by a number of different cell types, including immune, hematopoietic, vascular and epithelial cells, as well as tumor cells. It is a receptor for CD154. CD40 itself lacks intrinsic kinase activity and employs adaptor molecules to mediate its effects on processes such as proliferation, survival and, most importantly, immune responses. It has been shown that CD154–CD40 ligation activates nuclear factor NF-kappa-B (NF-κB) and c-Jun *N*-terminal kinase (JNK) signaling, and CD40 engagement induces tumor regression via an indirect effect of immune activation and a direct cytotoxic effect on the tumor. Various anti-CD40 antibodies have reached clinical evaluation in patients with chronic lymphocytic leukemia and other hematological malignancies. Moreover, studies have shown that the number of $CD40^+$ macrophages is significantly

elevated in both histologically inflamed and noninflamed colon and ileum of children with IBD. Antagonism of CD40 may therefore control the inflammation observed in Crohn's disease (19-23).

Chemokine receptor CCR9

CCR9 is a seven-transmembrane G-protein coupled receptor (GPCR) also known as CDw199 (cluster of differentiation w199) of the β-chemokine family that binds CCL25 (TECK). CCR9 has been shown to be differentially expressed by T lymphocytes of the small intestine and colon. This suggests that thymocyte recruitment and development may permit functional specialization of immune responses in different segments of the GI tract. Studies have demonstrated that the CCL25/CCR9 chemokine ligand/receptor pair is involved in small bowel immunity and inflammation. Aberrant expression of CCL25 in the small bowel has been observed in Crohn's disease, as well as an increased frequency of $CCR9^+$ T cells in the peripheral blood of patients with Crohn's disease and celiac disease. CCR9 antagonists may therefore be effective in the treatment of small-bowel Crohn's disease (24, 25).

Dihydroorotate dehydrogenase

Dihydroorotate dehydrogenase (EC 1.3.3.1) is a mitochondrial enzyme that catalyzes the fourth step in the de novo biosynthesis of pyrimidine-containing ribonucleotides; it catalyzes the ubiquinone-mediated oxidation of dihydroorotate to orotate. As rapidly proliferating human T cells have an exceptional requirement for de novo pyrimidine biosynthesis, small-molecule dihydroorotate dehydrogenase inhibitors constitute an attractive therapeutic approach to autoimmune diseases, immunosuppression and cancer. Inhibition of this enzyme would prevent T- and B-cell proliferation, and thus inflammatory responses, and may be beneficial in the treatment of Crohn's disease (26, 27).

Dual specificity mitogen-activated protein kinase kinase (MEK)

MEK (EC 2.7.12.2) is a kinase enzyme and a member of the mitogen-activated protein (MAP) kinase signal transduction cascade, where it lies upstream of MAP kinase and stimulates the enzymatic activity of MAP kinase. MAP kinases, also known as extracellular signal-regulated kinases (ERKs), are activated by a wide variety of extracellular signals and thus serve as an integration point for multiple biochemical pathways. They are activated via rapid phosphorylation on threonine and tyrosine residues. The MAP kinase signaling cascade is initiated by extracellular signaling, which activates (i.e., phosphorylates) MAP kinase kinase kinase (MAP3K). Activated MAP3K phosphorylates MEK, which then activates MAP kinase. In Crohn's disease, macroscopically noninflamed colon contributes to diarrhea via impaired epithelial sodium channel-mediated sodium absorption, and studies have shown that therapeutic inhibition of MEK1/2 restores electrogenic sodium absorption. Thus, inhibition of MEK could be an effective strategy for the treatment of the chronic inflammation and diarrhea seen in Crohn's disease (28-30).

Glucagon-like peptide 2 (GLP-2) receptor

The GLP-2 receptor is the GPCR for GLP-2, a GI peptide that promotes mucosal growth and the activity of intestinal brush border enzymes and delays gastric transit, allowing increased intestinal absorption. This receptor mediates signal transduction via activation of adenylyl cyclase. GLP-2 is an endocrine peptide derived from the posttranslational processing of proglucagon in the intestine. GLP-2 and the structurally related GLP-1 are derived from the same proglucagon precursor, and both peptides are produced and nutrient-dependently secreted by the enteroendocrine L cells of the small and large intestine. Whereas GLP-1 regulates pancreatic endocrine function and gastric motility, GLP-2 is trophic to the intestinal mucosal epithelium via stimulation of crypt cell proliferation and reduction of enterocyte apoptosis. GLP-2 plays an essential role in the regulation of mucosal morphology, function and integrity. GLP-2 receptor agonists have been shown to have positive effects on intestinal barrier function, splanchnic perfusion and mucosal healing in Crohn's disease and other intestinal disorders (31-36).

Integrin $\alpha_4\beta_1$ (VLA-4)

VLA-4 is an α_4 integrin also known as CD49d/CD29. It is a key cell-surface receptor expressed on leukocytes (i.e., lymphocytes, monocytes, mast cells, macrophages, natural killer [NK] cells, dendritic cells, basophils and eosinophils, but not neutrophils) and is involved in both adhesion and T-cell costimulation. It binds to both vascular cell adhesion protein 1 (V-CAM 1) expressed on cytokine-stimulated endothelial cells and the connecting segment (CS-1) domain of fibronectin, an extracellular matrix (ECM) protein. During inflammatory reactions, VLA-4 regulates cellular adhesion and leukocyte migration into tissues through activation of cell–cell and cell–matrix interactions. $CD4^+$ T cells are essential for the development and progression of Crohn's disease and studies have shown that pathogenic $CD4^+$ T cells use the $\alpha_4\beta_7$/MAdCAM-1 pathway, alternatively engaging VLA-4 and L-selectin to recirculate to the chronically inflamed small intestine. Thus, antagonism of VLA-4 is an attractive strategy for the treatment of chronic inflammatory diseases such as IBD, including Crohn's disease, as well as psoriasis and multiple sclerosis (21, 37, 38).

Integrin $\alpha_5\beta_1$ (VLA-5)

VLA-5 (also known as CD49e/CD29) is a member of a large family of widely expressed transmembrane receptors for ECM and plasma proteins. Like other integrins, $\alpha_5\beta_1$ is comprised of two noncovalently linked subunits (α_5 and β_1) that span the plasma membrane and interact with the actin cytoskeleton and focal adhesion kinase (FAK) complex through their cytoplasmic domains, which are highly conserved and provide specificity of interactions and fine-tuning of subsequent downstream signaling. A characteristic feature of all integrins, including $\alpha_5\beta_1$, is the ability to transmit signals bidirectionally, both inside-out and outside-in, regulating the processes of cell survival, growth and motility. Integrin $\alpha_5\beta_1$ interacts with various ECM proteins, including fibronectin, vitronectin and those that contain the RGD recognition sequence, and its expression has been found to be upregulated in newly formed blood vessels. Cells that overexpress $\alpha_5\beta_1$ are more resistant to apoptosis and express higher levels of Bcl-2, suggesting that $\alpha_5\beta_1$ has survival-supporting activity that is likely mediated by FAK, phosphatidylinositol 3-kinase (PI3K)/Akt and calcium/calmodulin-dependent protein kinase type IV (CaMK IV) signaling pathways. Neoangiogenesis is a critical component of chronic inflammatory dis-

orders such as Crohn's disease and inhibition of angiogenesis has been shown to be an effective treatment in animal models of experimental colitis. Antiangiogenic strategies may therefore be an effective therapeutic strategy for the treatment of Crohn's disease (21, 38, 39).

Integrin $\alpha_4\beta_7$ (LPAM-1)

LPAM-1 is an α_4 integrin (also known as CD49d/beta7) composed of the α_4 and β_7 subunits which form a lymphocyte-homing receptor that mediates lymphocyte attachment within the ECM by adhering to the CS-1 site of fibronectin. It belongs to the Ig superfamily and is found on the majority of peripheral lymphocytes and subsets of thymocytes and bone marrow cells (including mast cell progenitors). LPAM-1 binds V-CAM 1 (CD106), MAdCAM-1 and fibronectin, and plays an important role in lymphocyte adhesion and helps direct the migration of blood lymphocytes to the intestine and associated lymphoid tissues. Inflammation leading to tissue damage and disease is mediated in part by the α_4 integrins $\alpha_4\beta_1$ and $\alpha_4\beta_7$, which are expressed on the leukocyte cell surface. Studies have shown that the development of intestinal inflammation is dependent on β_7 integrin-mediated T-lymphocyte recruitment. Inhibition of leukocyte trafficking via antagonism of the α_4 integrin is a validated therapeutic approach for the treatment of inflammatory diseases such as IBD and multiple sclerosis, and monoclonal antibodies specific for α_4 integrins or their cell adhesion molecule (CAM) ligands can moderate inflammation in animal models, suggesting that such inhibitors may be useful for treating human inflammatory diseases such as Crohn's disease (21, 24, 40).

Integrin $\alpha_v\beta_3$ (vitronectin receptor)

Integrin $\alpha_v\beta_3$ is a member of a large family of transmembrane receptors for ECM and plasma proteins and is composed of two noncovalently linked subunits (α_v and β_3) that span the plasma membrane. Integrin $\alpha_v\beta_3$ interacts with various ECM proteins, including fibronectin, osteopontin, tenascin and vitronectin. Upon ligand binding, these subunits interact with the actin cytoskeleton and FAK complex through their cytoplasmic domains. It is known that endogenous insulin-like growth factor I (IGF-I) regulates intestinal smooth muscle growth by concomitantly stimulating proliferation and inhibiting apoptosis, and IGF-I-stimulated growth is augmented by the integrin $\alpha_v\beta_3$ ligands vitronectin and fibronectin. IGF-I expression in smooth muscle is increased in both experimental colitis and Crohn's disease. Thus, the smooth muscle hyperplasia and stricture formation observed in the chronically inflamed intestine may in part be due to upregulation of endogenous IGF-I and ligands of integrin $\alpha_v\beta_3$ that mediate the increase in smooth muscle cell proliferation and the decrease in apoptosis. Integrin $\alpha_v\beta_3$ has also been established as a proangiogenic factor that facilitates endothelial cell motility and migration. Thus, antagonists of $\alpha_v\beta_3$ may be effective in the treatment of Crohn's disease (41, 42).

Interleukin-12 (IL-12)

IL-12 is a heterodimeric cytokine that promotes cell-mediated immunity by facilitating type 1 helper T (Th1) lymphocyte responses, including the production of interferon gamma by both T cells and NK cells, potentiating the lytic activity of NK cells and boosting specific cytolytic T-lymphocyte responses. IL-12 has shown potent therapeutic effects in various cancers and infectious diseases, including certain viral infections. Overproduction of this inflammatory cytokine may be involved in autoimmune insulitis, type 1 diabetes, rheumatoid arthritis, psoriasis, multiple sclerosis and IBD. Thus, antagonism of IL-12 may be beneficial in the treatment of Th1-related autoimmune or immunological disorders such as Crohn's disease (43-46).

Interleukin-23 (IL-23)

IL-23 is a heterodimeric cytokine composed of a unique p19 subunit and the p40 subunit component of IL-12. It is secreted by activated dendritic cells, glial cells and macrophages, and binds to memory T cells, NK cells, macrophages and dendritic cells. In particular, this cytokine is suspected to be involved in the activation and maintenance of the Th17 subset of inflammatory T cells. It promotes upregulation of the matrix metalloproteinase MMP-9, increases angiogenesis and reduces $CD8^+$ T-cell infiltration. The IL-12 family of cytokines (IL-12, IL-23, IL-27) plays a critical role in the differentiation of Th1 cells. Moreover, it has been hypothesized that the autoimmune actions of IL-12 are attributable to IL-23. Overexpression of IL-23 and/or IL-12 or a defect in their receptors may be involved in conditions such as rheumatoid arthritis, psoriatic arthritis, psoriasis, ankylosing spondylitis, multiple sclerosis and Crohn's disease. Patients with Crohn's disease have been shown to have a significantly increased number of intestinal $CD14^+$ macrophages compared to normal control subjects, and these cells produce larger amounts of IL-23 and TNF-α compared to normal controls or patients with ulcerative colitis. Moreover, genomic studies conducted in patients with Crohn's disease have identified the IL-23 pathway as playing a predominant role in this disorder. Thus, monoclonal antibodies directed against both IL-12 and IL-23 may be effective treatment options for Crohn's disease (47-52).

Janus kinase 3 (JAK3)

JAK3 is a large cytoplasmic cellular protein that is a member of the Janus family of tyrosine-protein kinases which also includes JAK1, JAK2 and TYK. JAKs, via tyrosine phosphorylation, activate cytokine-mediated latent cytoplasmic signal transducers and activators of transcription (STATs). Several cytokines critical for immune cell development and homeostasis (IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21) that bind to receptors sharing the cytokine receptor common gamma chain (Gamma-C), have emerged as crucial entities regulating immune function. Following cytokine binding to cell-surface receptors, signal transduction to the nuclei is achieved via activation of JAKs, which consequently phosphorylate the cytokine receptor, creating a site for docking of STATs. STATs are phosphorylated by JAKs and dimerization occurs, followed by transport to the nucleus, DNA binding, and ultimately, transcriptional modulation. In contrast to JAK1, JAK2 and TYK2, which are used by a variety cytokine receptors and are ubiquitously expressed, JAK3 expression is relatively restricted and is only used by cytokine receptors containing Gamma-C. High levels of JAK3 are found in NK cells and thymocytes and it is inducible in T cells, B cells and myeloid cells, but is not expressed in resting T cells. Interestingly, studies have shown that patients carrying mutations in the gene encoding JAK3 present the same $T^-B^+NK^-$SCID (severe combined immunodeficiency) phenotype. Moreover, activation of STAT4 and STAT3 in T cells, which is mediat-

ed by JAKs, appears to play a key pathogenic role in Crohn's disease. JAK3 is a potentially effective and selective target for the development of novel immunosuppressive agents for the treatment of various autoimmune diseases such as rheumatoid arthritis, psoriasis and Crohn's disease (53-55).

Nuclear factor NF-kappa-B (NF-κB)

NF-κB is a protein transcription factor and intracellular mediator of the inflammatory cascade involved in the generation of adhesion molecules (ICAM-1, V-CAM 1), inducible nitric oxide synthase (iNOS), cyclooxygenase COX-2, cytokines (e.g., IL-1β, IL-2, TNF-α, IL-6, interferon gamma) and chemokines (e.g., IL-8). Other genes which are regulated by NF-κB include those encoding the IL-2 receptor, the IL-12 p40 subunit and c-Myc. NF-κB provides a mechanistic link between inflammation and cancer, controlling the ability of preneoplastic and malignant cells to resist apoptosis-based tumor surveillance mechanism and regulating tumor angiogenesis and invasiveness. NF-κB activity is closely associated with the I-kappa-B-kinase complex (IKK), and aberrant or constitutive NF-κB activation has been detected in many human malignancies. It has also been reported that constitutive activation of the tyrosine-protein kinase receptor FLT3 is responsible for IKK activation. Moreover, TNF activation results in NF-κB activation and plays a role in inflammation, and is an important signaling factor for cytokines that appear to participate in several pathological conditions, such as multiple sclerosis, Parkinson's disease, depression and IBD. NF-κB has been implicated in various aspects of neuroplasticity, including long-term potentiation and cellular apoptosis and differentiation. Enhanced NF-κB activity is involved in the pathology of Crohn's disease and ulcerative colitis, and studies have demonstrated enhanced processing of the NF-κB precursor p105 and degradation of NF-κB inhibitor alpha (IκBα) by immunoproteasomes isolated from the mucosa of patients with Crohn's disease. NF-κB has been identified as an important target for therapeutic intervention in IBD since it plays a central role in regulating inflammatory responses in these patients. NF-κB activation inhibitors may therefore be effective for suppressing inflammation seen in Th17-mediated diseases such as rheumatoid arthritis, psoriasis and Crohn's disease (56-58).

Pregnane X receptor (PXR)

The PXR, also known as nuclear receptor subfamily I group I member 2, is a nuclear receptor and transcription factor that responds to the presence of toxic substances by upregulating the expression of proteins involved in the detoxification and clearance processes of the body. When activated (e.g., binding of endogenous and exogenous ligands including steroids, antibiotics, antimycotics, bile acids, hyperforin), it forms a heterodimer with the retinoid X receptor (RXR) and binds to hormone response elements on DNA, resulting in gene expression. The primary targets of PXR activation include induction of genes involved in the detoxification process in the liver and intestine, such as phase I oxidative enzymes (e.g., CYP3A4), phase II conjugating enzymes (e.g., glutathione *S*-transferase) and phase III transport uptake and efflux proteins (e.g., OATP-2, MDR1). The PXR gene (*NR1I2, PXR*) is associated with an increased risk for IBD. MDR1 expression is strongly decreased in inflamed intestinal epithelia of patients with GI disorders such as Crohn's disease and ulcerative colitis, and these low MDR1 levels may aggravate intestinal inflammation. PXR agonists could therefore be effective in the treatment of Crohn's disease (59-61).

P-selectin glycoprotein ligand 1 (PSGL-1, CD162)

CD162, also known as P-selectin glycoprotein ligand 1 (PSGL-1), is a dimeric sialomucin that binds to P-, E- and L-selectins. It mediates the adhesive interactions between circulating leukocytes and platelets by binding to P-selectin, between circulating leukocytes and endothelial cells by binding to E-selectin, and between leukocytes by binding to L-selectin. It has been found to be overexpressed in the granulocytes of patients with allergic asthma and in the granulocytes, monocytes and lymphocytes of patients with chronic obstructive pulmonary disease (COPD), playing a role in inflammation in these diseases and in others such as IBD. The interaction between E-selectin and integrins may facilitate the movement of leukocytes through the endothelium to inflammatory foci. Crohn's disease and other chronic intestinal disorders are characterized by immune dysregulation and leukocyte recruitment into the GI tract. CAMs mediate the extravasation of leukocytes and their accumulation in inflamed intestinal mucosa. Thus, monoclonal antibodies against CD162 may be effective in the treatment of Crohn's disease (38, 62, 63).

TNF-α

TNF-α (also known as cachectin) is a proinflammatory cytokine and a member of the TNF family of cytokines that is released by activated macrophages and lymphocytes. It acts via receptors belonging to the TNF family of receptors, among which TNF-R1 and TNF-R2 trigger several signal transduction pathways, resulting in the activation of transcription factors such as NF-κB and c-fos/c-jun. TNF-R1 (also known as CD120a, p55/60) is expressed in most tissues and is fully activated by both the membrane-bound and soluble trimeric forms of TNF. TNF-R2 (also known as CD120b, p75/80), however, is found only in cells of the immune system and is activated by the membrane-bound form of the TNF homotrimer. Activated factors induce the transcription of antiapoptotic, proliferative, immunomodulatory and inflammatory genes. NF-κB is the major survival factor in preventing TNF-α-induced apoptosis. TNF-α is also a crucial cytokine in the establishment and maintenance of inflammation in multiple autoimmune diseases. Elevated levels of TNF-α are found in a wide range of diseases, including the chronic inflammatory conditions rheumatoid arthritis, psoriasis and Crohn's disease. Inhibition of the TNF-α signaling pathway (e.g., TNF-α blockers, blockers of p38, JNK and/or ERK kinases, antagonists of transcription factor NF-κB activation) is an attractive therapeutic strategy for the treatment of Crohn's disease, as well as multiple sclerosis, psoriasis, psoriatic arthritis, uveitis, sarcoidosis, Behçet's syndrome, graft versus host disease and ankylosing spondylitis (21, 22, 64).

Zonulin

Zonulin is a protein homologous to zona occludens toxin (Zot) of *Vibrio cholerae* that modulates intestinal permeability by inducing tight junction disassembly. It is upregulated in several autoimmune diseases such as IBD. The GI tract, in addition to digestive and absorptive functions, also regulates the trafficking of macromolecules between the environment and the host through a barrier

mechanism to prevent harm and minimize inflammation. This trafficking is safeguarded by intercellular tight junctions, the physiological modulation of which is mediated by zonulin, among other proteins. Zonulin regulates intestinal permeability and the zonulin pathway has been exploited to deliver drugs, macromolecules or vaccines that normally would not be absorbed through the GI mucosal barrier. However, if zonulin becomes chronically upregulated and there is a dysfunctioning of trafficking of macromolecules (i.e., increased intestinal permeability) and excessive flow of non-self antigens in the intestinal submucosa, this may provoke intestinal and extraintestinal autoimmune disorders in genetically susceptible individuals. Increased intestinal epithelial permeability precedes clinical relapse by as much as one year in clinically asymptomatic Crohn's disease patients. This suggests that a permeability defect possibly due to activation of the zonulin pathway may be involved in the early steps of the pathogenesis of IBD, while the production of cytokines, including interferon gamma and TNF-α, secondary to inflammation, perpetuates increased intestinal permeability by reorganizing the tight junction proteins ZO-1, JAM 1, occludin, claudin-1 and claudin-4. It has been suggested that abnormal intestinal barrier function is a genetic trait involved in the pathogenesis of IBD. Moreover, zonulin upregulation can be detected in the acute phase of IBD and although serum levels decrease, they remain higher than normal once the inflammatory process subsides with treatment. A zonulin receptor antagonist could therefore be therapeutically effective in the acute phase of Crohn's disease (5, 65-67).

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