Pathogenic angiogenesis in IBD and experimental colitis: new ideas and therapeutic avenues

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Chidlow JH Jr, Shukla D, Grisham MB, Kevil CG. Pathogenic angiogenesis in IBD and experimental colitis: new ideas and therapeutic avenues. *Am J Physiol Gastrointest Liver Physiol* 293: G5–G18, 2007. First published April 26, 2007; doi:10.1152/ajpgi.00107.2007.—Angiogenesis is now understood to play a major role in the pathology of chronic inflammatory diseases and is indicated to exacerbate disease pathology. Recent evidence shows that angiogenesis is crucial during inflammatory bowel disease (IBD) and in experimental models of colitis. Examination of the relationship between angiogenesis and inflammation in experimental colitis shows that initiating factors for these responses simultaneously increase as disease progresses and correlate in magnitude. Recent studies show that inhibition of the inflammatory response attenuates angiogenesis to a similar degree and, importantly, that inhibition of angiogenesis does the same to inflammation. Recent data provide evidence that differential regulation of the angiogenic mediators involved in IBD-associated chronic inflammation is the root of this pathological angiogenesis. Many factors are involved in this phenomenon, including growth factors/cytokines, chemokines, adhesion molecules, integrins, matrix-associated molecules, and signaling targets. These factors are produced by various vascular, inflammatory, and immune cell types that are involved in IBD pathology. Moreover, recent studies provide evidence that antiangiogenic therapy is a novel and effective approach for IBD treatment. Here we review the role of pathological angiogenesis during IBD and experimental colitis and discuss the therapeutic avenues this recent knowledge has revealed.

inflammation; T cells; growth factors; adhesion molecules; antiangiogenesis

ANGIOGENESIS PLAYS AN IMPORTANT role in many chronic inflammatory diseases including but not limited to diabetic retinopathy, atherosclerosis, rheumatoid arthritis, hypercholesterolemia, and psoriasis. It has been suggested that the angiogenic components of these diseases contribute to and exacerbate disease conditions (26, 31). Recent findings, both clinical and experimental, indicate that angiogenesis also plays a crucial role in the inflammatory bowel diseases (IBD), consisting of Crohn's disease (CD) and ulcerative colitis (UC) (31, 37, 58, 131, 155). Development of new vasculature during chronic inflammation may play a negative "pathological" role by contributing to increased inflammatory responses due to dysfunctional new vessel architecture and increases in the recruitment of inflammatory cell types. Importantly, recent data have shown that angiogenic inhibition during chronic inflammatory diseases attenuates further inflammation and disease pathology (31, 37, 51). As such, expanding our knowledge of how pathological angiogenesis occurs during IBD will further our ability to treat patients with these diseases.

IBD Pathogenesis

CD and UC are idiopathic inflammatory disorders of the intestine and/or colon in which patients suffer from rectal bleeding, severe diarrhea, abdominal pain, fever, and weight loss. Although the specific causes of IBD are poorly understood, the pathological natures of CD and UC have been extensively defined. CD primarily involves the small and large bowel, whereas UC is confined to the colon. Histological examination of biopsies obtained from patients with active CD reveals the presence of large numbers of leukocytes such as polymorphonuclear leukocytes, lymphocytes, and monocytes in the intestinal and/or colonic interstitium resulting in granulomatous inflammation. Coincident with this inflammatory infiltrate is extensive transmural injury including edema, loss of goblet cells, decreased mucus production, crypt cell hyperplasia, erosions, and ulcerations. Active episodes of UC share many of the same histopathological characteristics observed in CD; however, the inflammation is confined to the mucosa. The broad pathological process of both diseases involves inflammation, ulceration, and subsequent regeneration of intestinal mucosa (49, 164). These episodes are often cyclical in nature, thereby resulting in periods of increased disease activity (flares) followed by days or weeks of quiescence.

The etiology of CD or UC has not been fully elucidated; however, there is growing clinical and experimental evidence to suggest that the initiation and progression of these inflammatory disorders involve complex interactions among genetic, immune, and environmental factors (10, 50, 72). A number of different studies, using a variety of immune-manipulated and genetically engineered animal models of chronic colitis, sug-

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gest that chronic gut inflammation may result from a dysregulated immune response to components of the normal gut flora (121, 123, 149, 157). This paradigm involves activated immune cells producing copious amounts of IFN-γ, IL-17, TNF-α, lymphotoxin-α, and IL-2 (Fig. 1), which promote production of large amounts of cytokines and reactive oxygen and nitrogen metabolites [e.g., superoxide, hydrogen peroxide, nitric oxide (NO)]. The net result of this uncontrolled production of Th1/Th17- and macrophage-derived inflammatory mediators is the activation of the microvascular endothelial cells to increase surface expression of endothelial cell adhesion molecules, thereby promoting the recruitment of additional leukocytes (e.g., polymorphonuclear leukocytes, monocytes, macrophages) into the gut (Fig. 1). In addition their role as proinflammatory mediators, many of these cytokines and mediators possess potent proangiogenic properties. The fact that vessel density is increased in active but not quiescent CD (143), coupled to the above-mentioned reports demonstrating the production of a variety of proangiogenic factors, suggests that angiogenesis may play an important role in the pathogenesis of IBD. This review provides an overview of the biology and pathophysiology of angiogenesis and presents evidence to suggest that "pathological" angiogenesis may act to perpetuate chronic gut inflammation.

Involvement of the Microvasculature in IBD

Vascular changes in IBD were noted as early as 1954 and throughout the late 1950s and 1960s; however, descriptions of these vascular changes and the interpretations of what they meant varied widely (8, 78, 125, 164). In 1970, Brahme and Lindstrom (21) reported increases in vascularity in active Crohn's disease which they showed by radiography of vascular castings. Recent forays into human and experimental colitis have further indicated an important role for the microvasculature in IBD (58, 79, 85). Increased microvascular density in CD and UC has been shown clinically, and our work corroborates this through PECAM-1/CD31 staining of the microvasculature in CD specimens where vessel density is significantly increased in the mucosal and submucosal tissue layers with a total increase in vascular density being similar to that observed in experimental colitis (Fig. 2) (31, 58, 92, 143). Interestingly, microvascular dysfunction during IBD shows a temporal relationship with tissue pathology based on observed microscopic alterations in tissue morphology (95). These data provide evidence that vascular changes, in the form of angiogenesis, are critical to the disease process.

The development of new blood vessels is a necessary part of life from it earliest stages. Vasculogenesis is the initial process
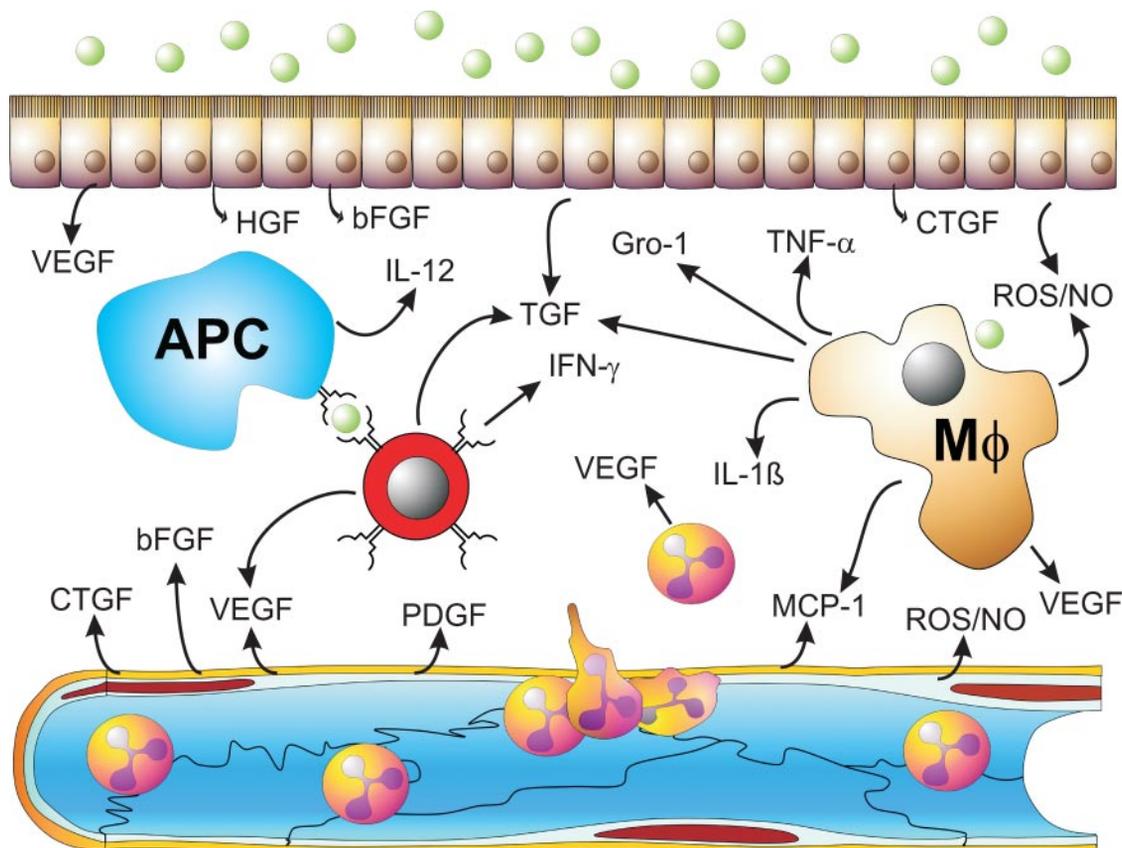

Fig. 1. Release of angiogenic mediators from inflammatory cells. Invading and resident inflammatory cell types produce and release various mediators of inflammation and angiogenesis during inflammatory bowel disease (IBD) and experimental colitis. Infiltrating neutrophils (light purple), monocytes/macrophages (light brown), T cells (red), and antigen presenting cells (APC; blue) play a large role in this process by secreting cytokines, growth factors, and chemokines as stimulators of angiogenesis. HGF, hepatocyte growth factor; bFGF, basic fibroblast growth factor; Gro-1, growth-regulated oncogene-1; CTGF, connective tissue growth factor; ROS/NO, reactive oxygen species/nitric oxide; PDGF, platelet-derived growth factor; MCP-1, monocyte chemoattractant protein-1; Mφ, macrophage.
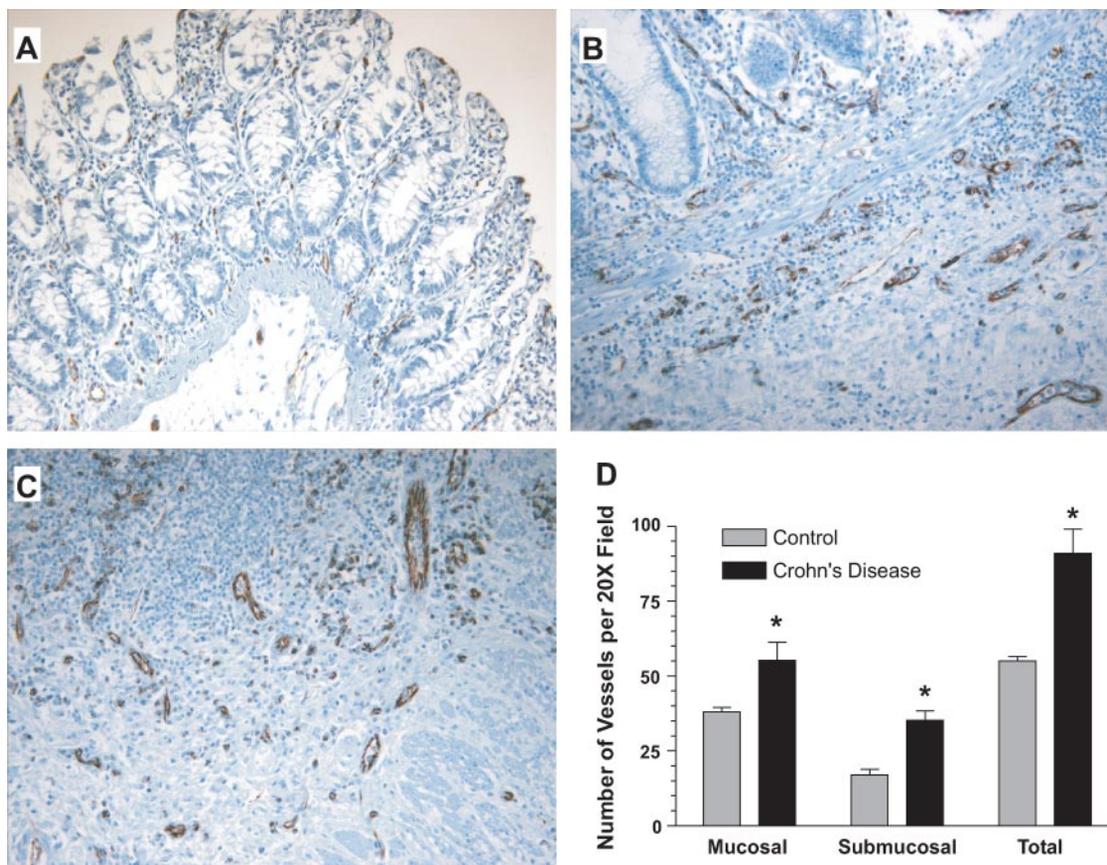
Fig. 2. Platelet endothelial cell adhesion molecule-1 (PECAM-1) staining of colon cross sections taken from Crohn's disease (CD) patients. Tissue taken from the colons of patients with CD was sectioned and stained for PECAM-1/CD31 (brown) and counterstained with hematoxylin (blue). *A*: CD31 staining of normal human colon tissue. *B* and *C*: representative CD31 staining of colon tissue from active Crohn's disease patients. Total vessel numbers were counted in the mucosa, submucosa, and in the whole tissue (*D*). Examination of these data revealed significant increases in vascular density in both the mucosa and submucosa separately and an overall increase in total vascular density in these colons. Photomicrograph magnification is $\times 200$.

during embryonic development by which the blood supply for the forming organism is created. After vasculogenesis, the formation of new vessels is known as angiogenesis and is critical to wound healing and development of the corpus luteum during the female reproductive cycle. New vessels are produced by one of two identified vascular growth phenomena. The first, sprouting, is the process whereby new vessels occur as offshoots of existing vessels and grow outward from these vessels, and the second, known as intussusceptive growth, is the process whereby existing vessels divide into two distinct parallel vessels through a multistep process. Normal wound healing angiogenesis, known as physiological angiogenesis, is closely controlled by multiple growth and tissue factors resulting in minimal changes in microvascular permeability, proteolysis, and inflammation. However, abnormal or pathological angiogenesis, such as that observed in IBD, is characterized by its abnormal vasculature, which exhibits torturous architecture, increased permeability, and increased inflammatory and thrombogenic potential.

An understanding of microvascular changes during angiogenesis in inflamed tissues is necessary to clarify the role of the microvasculature during chronic inflammation. It is thought that the different microvascular segments, arterioles, capillaries, and venules all have a specific roles in this process through their various interactions with angiogenic mediators (86). Im-

portantly, venules act as the site of most activity in the development of inflammation and through recruitment of cell types that produce angiogenic mediators. The multistep process of angiogenesis begins with the production of various angiogenic cytokines, which are released from inflamed tissue and bind endothelial cell surface receptors initiating intracellular signaling that in turn causes dilation of vessels, increased vascular permeability, and degradation of the underlying basement membrane. Angiogenic chemokines and cytokines then stimulate endothelial cell proliferation and directional migration. Various integrins, matrix metalloproteinases (MMPs), and additional mediators are then involved in remodeling the extracellular matrix (ECM) and incorporating migrating endothelial cells within the reorganizing ECM. Endothelial cells that have migrated into the ECM then undergo the processes of tube or lumen formation and junctional complex maturation as new vessels begin to form. The vascular tubes produced at this stage will then anastomose with other sprouting vessels and undergo arteriolar-venular differentiation. Once this has occurred, endothelial cell proliferation and cell migration cease and smooth muscle cells and pericytes attach to stabilize the new vasculature, thus completing the process.

Until recently, the microvascular changes that occur during IBD have not been closely investigated, and we are only beginning to understand their involvement in the disease pro-

cess. Our laboratory has recently determined that differential regulation of pro- vs. antiangiogenic gene expression dictates this phenomenon by creating imbalances between these regulatory factors (i.e., upregulation of pro- over antiangiogenic factors or relative downregulation of antiangiogenic factors) (31). Evidence indicates that some of the upregulated angiogenic factors involved in colitis may prevent the maturation of vessels, contributing to the pathological nature of this angiogenesis. Recently, angiostatin and endostatin have been shown to be involved during IBD, and it is possible that they are involved in prevention of vessel maturation and ulcer healing (131). For example, angiostatin production by MMPs has been shown to inhibit vessel maturation (36), and inhibition of pericyte stabilization of the vasculature results in endothelial cell hyperplasia and abnormal formation (59). These occurrences during IBD could contribute to a pathological phenotype of angiogenesis. The assumption that wound healing angiogenesis can occur in the form of ulcer repair during remission of IBD suggests a role for physiological angiogenesis in IBD and may also represent a return to normal regulation of angiogenic mediators during disease remission. However, the majority of tissue alterations that occur during IBD are not fully corrected in remission (79). Thus the role of the microvasculature in inflammatory angiogenesis is critical to the process of chronic inflammation, and understanding the differential regulation of angiogenic factors involved will provide insight into IBD pathology.

Experimental Colitis and Angiogenesis

Several models of IBD have been developed and only recently have been used to study different components of angiogenesis. For example, the dextran sulfate sodium (DSS), trinitrobenzenesulfonic acid (TNBS)-induced colitis, the CD4+CD45RB^{high} T cell transfer, and the IL-10 gene targeted knockout models have been evaluated. These models are best described as being either T cell dependent or T cell independent because this determination coincides with the aspects of disease best studied in each model. Both the CD4+CD45RB^{high} and IL-10 knockout models are largely T cell dependent whereas the DSS and TNBS models are largely T cell independent. The CD4+CD45RB^{high} model was developed by Morrissey et al. (100, 101) in 1993; here colitis is elicited by reconstitution of immunodeficient mice with an immunologically naive population of T cells termed CD4+CD45RB^{high} cells (122). This model has been used for investigating specific mechanisms of T cell-mediated colitis and we have recently shown that these mice have an increased angiogenic index (AI) representing increased vascular density (31, 120, 124). IL-10 knockout colitis is a genetic model developed by Kuhn and colleagues (82) that is characterized by involvement of the entire intestinal tract. This model has been useful for establishing the need for strict regulation of the mucosal immune response and for identifying key components in gut immune regulation (119). The T cell-independent DSS and TNBS models are chemically induced by administering 3–5% DSS in the drinking water or by injecting a bolus of TNBS in 30–50% ethanol in the anus, respectively. The DSS model is an erosive, neutrophil-dependent model developed by Okayasu and colleagues (106). The DSS model has been used extensively to investigate the role of various leukocytes during

severe colitis and we have seen increased vascular density during the progression of disease (31, 119). Morris et al. (99) developed the TNBS model, and it is particularly useful for studying biochemical inflammatory pathways and performing antigen-specific studies (119). Together, these models have played a crucial role in increasing our knowledge of IBD and manifest significant angiogenic responses during experimental colitis.

There are a plethora of mediators involved in both physiological and pathological angiogenesis including vasoactive agents, growth factors/cytokines, chemokines, adhesion molecules and their associated ligands, and ECM and signaling molecules (Fig. 1). These mediators are produced by a variety of cell types including vascular cells (endothelial cells, smooth muscle cells), epithelial cells, inflammatory cell types (T cells, leukocytes), ECM components, and mesenchymal and nerve cells (Fig. 1). Regulation of these factors compared with one another is integral in differentiating between a physiological vs. pathological response, because it is now understood that angiogenesis plays a critical role in the pathology of IBD and its experimental models. Recent data shown by several groups, including ours, demonstrates a significant increase in AI in models of experimental colitis (31, 37). AI is increased in the colons of the CD4+CD45RB^{high} and both the acute and chronic 3% DSS models about twofold (31). Additionally, similar increases in vascular density have been observed in the IL-10$^{-/-}$ model (38). Gene array studies of experimental colitis tissue reveals differential expression of important angiogenic mediators such as vascular endothelial growth factor-A (VEGF-A), basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), and others and show an overall pattern of upregulation of proangiogenic genes concomitant with downregulation of antiangiogenic genes in the CD4+CD45RB^{high} model (31). Interestingly, data from the DSS model indicate that it may involve a preferential overall downregulation of antiangiogenic mediators compared with proangiogenic protein upregulation (31). These angiogenic factors play a myriad of critical roles in the development and stabilization of new vasculature as discussed above. Importantly, recent studies have shown that impairment of the angiogenic response during experimental colitis results in decreased AI and a decrease in overall tissue pathology and disease symptoms (31, 37). These data parallel observations made in human IBD and make antiangiogenic therapy a prime target for IBD treatment.

Relationship Between Inflammation and Angiogenesis

Dysfunctional immune responses are known to be causative factors in IBD and experimental colitis as well as other chronic inflammatory diseases. During inflammation, cell signaling results in increased expression of adhesion molecules on the surface of endothelial cells, which facilitates leukocyte recruitment. Interactions between leukocytes and endothelium lead to leukocyte rolling along the endothelium, a process that results in the firm adhesion of leukocytes to the endothelial cells. Adherent leukocytes then emigrate into the surrounding tissue by transmigration across the endothelial layer. Once in the tissue, immune cells release several different mediators of both inflammation and angiogenesis, initiating chronic inflammation as observed in experimental colitis and IBD.

Mediators of chronic inflammation, both cellular (i.e., leukocytes and platelets) and biochemical (i.e., cytokine/chemokine), are potent stimuli for angiogenesis through varying mechanisms (28, 29, 83). For example, neutrophils, monocytes, and macrophages make large amounts of VEGF-A through stimulation of transcriptional induction and enhanced protein expression of VEGF-A as well as NO which is well known to upregulate the expression of VEGF-A (83, 111, 141). Several proinflammatory cytokines/chemokines, such as TNF-α, IL-1β, monocyte chemoattractant protein-1 (MCP-1), and growth-regulated oncogene-1 (Gro-1), are also released from infiltrating leukocytes, which activate endothelial cells, resulting in neovascularization (Fig. 1) (128, 140, 148). Moreover, infiltrated leukocytes produce ample proteolytic enzymes, such as MMPs, that facilitate angiogenic responses (Fig. 3) (15, 104, 136). Additionally, IFN-γ released from activated T cells interacts with and activates antigen presenting cells and macrophages to produce large amounts of cytokines such as TNF-α, IL-1β, MCP-1, Gro-1, IL-6, IL-8, IL-12, and IL-18 as well as reactive oxygen and nitrogen metabolites (128, 148). All of the factors mentioned above have been identified in IBD or experimental colitis, suggesting that inflammation may beget pathological angiogenesis in the colon.

Additionally, we have reported a high degree of correlation between histopathological damage and increases in vascular density in the colons of mice with both DSS and $CD4^+CD45RB^{high}$ colitis ($R^2 > 0.9$ in both models), suggesting that angiogenesis coincides with the inflammatory response (31). Recent studies from our laboratory have also focused on the pathogenic role of the leukocyte β2 (CD18) integrin family of proteins, consisting of CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1), CD11c/CD18, and CD11d/CD18, which are important for the recruitment of leukocytes during inflammation. These studies show that β2-integrin-deficient mice, which have impaired ability to recruit leukocytes, have a significantly decreased inflammatory response, greatly attenuated disease, and lower vascular density compared with wild-type mice, further solidifying the link between chronic inflammation and angiogenesis (1, 31, 113). The specific mechanisms of "inflammatory angiogenesis" are unknown in IBD; however, it is the imbalance that occurs between inflammatory cell-produced pro- and antiangiogenic mediators in experimental colitis that appears to lead to pathological angiogenesis.

Angiogenic Mediators in IBD and Experimental Colitis

Growth factors. Many growth factors and their receptors are upregulated and may be critical for angiogenesis during experimental colitis and IBD. These include VEGF-A, platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), bFGF, and TGF-β. All of these growth factors are known to be produced in the gastrointestinal tract and are upregulated during experimental colitis as well as in human disease (31). These growth factors act as cytokines stimulating endothelial growth and proliferation, as well as exerting various effects on other cell types. Thus defining the role of these growth factors in colitis should clarify differential regulatory mechanisms of pathological angiogenesis during IBD and provide additional options for inhibiting angiogenic activity during disease.
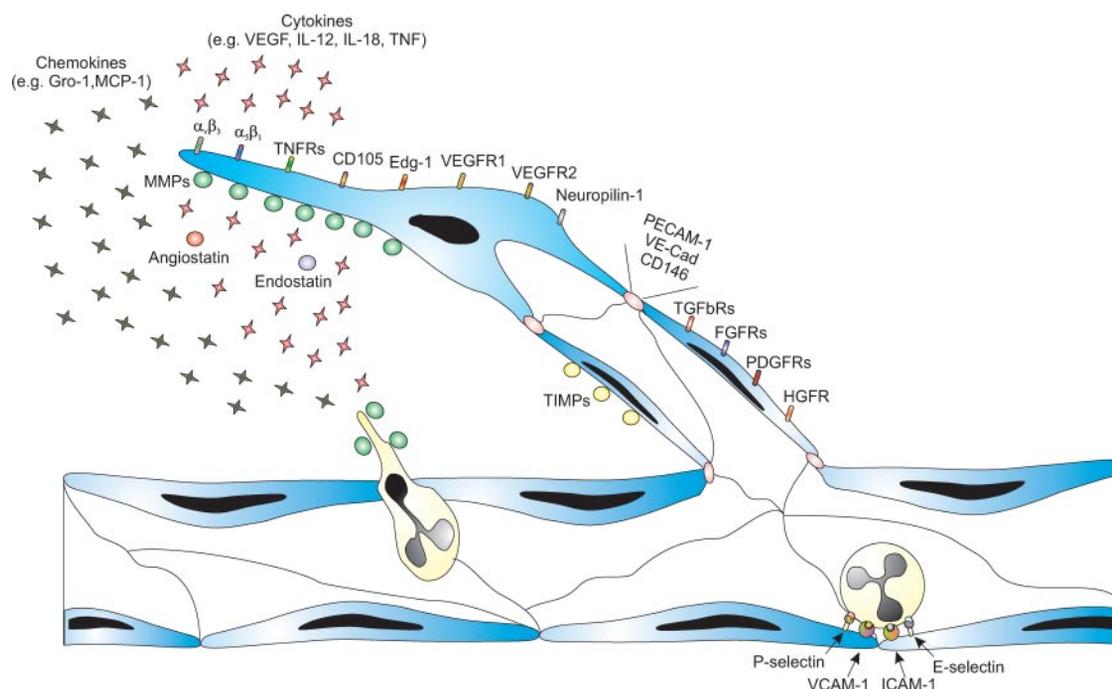

Fig. 3. Adhesion molecules, receptors, chemokines, cytokines, and other angiogenic mediators involved in IBD-associated angiogenesis. This figure depicts the general locale of adhesion molecules, growth factor, and cytokine receptors, cytokines, chemokines, matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), angiostatin, and endostatin during the colitis angiogenic response. Endothelial cells (blue) form a sprouting vessel as directed by the mediators depicted here. Chemokines (gray stars) and cytokines (red stars) are signaling from the extracellular matrix (ECM). MMPs (green) remodel the ECM to incorporate migrating endothelium. TIMPs (yellow) inhibit MMPs, stabilizing the matrix. Receptors (colored pills) interact with their ligands signaling for endothelial growth, proliferation, and migration as well as vascular maturation. Leukocytes (light yellow) interact with endothelial cell adhesion molecules as they adhere to the vessel wall and emigrate into the inflamed tissue to release angiogenic mediators. VE-Cad, vascular endothelial cadherin.

The involvement of VEGF-A in angiogenesis has been widely studied and is implicated in angiogenesis during colitis. VEGF-A is a potent proangiogenic factor that causes transient vasodilation through NO release, increased vascular permeability, and endothelial cell proliferation, directed migration, and differentiation (47). VEGF-A is part of a family of VEGFs: VEGF-A, -B, -C, and -D. VEGF-A and -B are involved in angiogenesis where VEGF-A is thought to play the major role in active growth and pathological angiogenesis (44, 46). VEGF-C and -D are involved in lymphangiogenesis and are complementary to the roles of VEGF-A and -B, although their involvement in IBD is not yet entirely clear (47). Many cell types produce VEGF-A during inflammation; these include stromal cells, neutrophils, platelets, monocytes, endothelial cells, epithelial cells, vascular smooth muscle cells, and activated T cells (47, 98). Throughout the process of angiogenesis VEGF-A plays multiple roles; it is important for initial tissue alterations needed for angiogenesis and is necessary until the new vessels are stabilized by pericytes. Increases in vascular permeability associated with VEGF-A are mediated by caveolae (22, 45) and may occur due to VEGF-A induction of fenestrae in the endothelium as shown by Roberts and Palade (127). In addition, VEGF-A causes junctional adhesion molecule-C expression to be upregulated and localized at cell junctions where VEGF-A has been shown to stimulate occludin and vascular endothelial cadherin (VE-cadherin), endothelial cell junctional molecules, phosphorylation leading to decreased occludin and VE-cadherin content at endothelial tight junctions, and increased vascular permeability (6, 73, 107). A major mediator of increased VEGF-A expression is HIF-1α, which is induced in response to hypoxia (33). The biological actions of VEGF-A are carried out through interaction with the receptors VEGFR-1 (Flt-1), VEGFR-2 (KDR), and neuropilin-1 (NP-1). Various effects of VEGF-A occur owing to differential binding with these receptors, resulting in signal pathway activation of MAPK pathways and many others.

There are four major isoforms of VEGF-A: $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$, which correspond to $VEGF_{120}$, $VEGF_{164}$, $VEGF_{188}$, and $VEGF_{205}$ in mice. These isoforms have differing binding properties and the exact roles of each during angiogenesis are not fully understood (46). Importantly, $VEGF_{164/165}$ is indicated as a major contributor to pathological angiogenesis and is highly active owing to its intermediate characteristics, existing both bound to the ECM and in soluble form with a heparin binding domain that increases its affinity for VEGFR-2 (47, 158). $VEGF_{165}$ binding of VEGFR-2 is enhanced by binding to NP-1 receptors, and this combination is a potent stimulator of angiogenic responses (47).

VEGF-A has been shown to be elevated in the tissue and serum of patients with CD and UC and in distal colon tissue from the $CD4^+CD45RB^{high}$ model of colitis (31, 70, 71). Specifically, Kanazawa et al. (70) and Kapsoritakis et al. (71) examined serum VEGF-A levels in CD and UC patients and found that VEGF-A is significantly elevated with active disease, but not in remission. However, platelets are major producers of VEGF-A and Kapsoritakis et al. indicate that ex vivo release of VEGF by platelets may give a false reading of VEGF levels. Similarly, Bousvaros et al. (18) showed that VEGF-A serum levels in children and young adults are elevated during active CD. Finally, Danese et al. (37) have also reported elevated levels of VEGF-A in mucosal tissue extracts from CD

and UC patients. Interestingly, one study found monocytes to be a major source of $VEGF_{165}$ in active IBD patients (57). Also to be noted, placenta-like growth factor is the evolutionary predecessor of VEGF and also upregulated and involved in angiogenesis during experimental colitis (31). All of these data demonstrate that VEGF-A expression is involved in IBD-associated angiogenesis and may be essential to disease pathology.

PDGF is a potent angiogenic mediator that is also upregulated during colitis and that is synthesized by many cell types in response to hypoxia, thrombin, and other cytokines and growth factors (3). PDGF is known to be a mitogen and chemoattractant for mesenchymal cells and a chemoattractant for neutrophils and monocytes. PDGF is implicated in angiogenesis as being important for recruitment of pericytes and vascular smooth muscle cells necessary for new blood vessel maturation through interaction with PDGFR-β (3, 88). Another growth factor, HGF, is secreted from stromal cells during inflammation and activated by HGF activator, which is activated by thrombin in injured tissues. This growth factor promotes proliferation, activation, and differentiation of intestinal endothelial and epithelial cells during angiogenesis and facilitates mucosal repair in experimental colitis (65, 151). Additionally, bFGF is a heparin-binding protein involved in intestinal inflammation and it promotes angiogenesis through endothelial cell proliferation, migration, and differentiation, as well as proliferation of mesenchymal cells (67, 154). Lastly, TGF-β is a pleiotropic angiogenic peptide growth factor that regulates proliferation, migration, differentiation, and proliferation in endothelial cells and ECM synthesis through signaling with its receptors TGFR-2 and -3 and is released by T cells during mucosal inflammation (17, 150). Our data show all of these growth factors to be upregulated during experimental colitis (31), and these mediators are all crucial in directing endothelial cells to create new vasculature.

These growth factors have been examined in human disease by several groups indicating their involvement in pathological angiogenesis. Human IBD patients serum and mucosal tissue levels of bFGF were reported to be elevated in both active CD and UC, whereas TGF-β serum levels were elevated in those with active CD (37, 70). Additional studies found elevated plasma bFGF in UC patients and elevated serum bFGF in children with CD (19, 154). Saito et al. (130) also found that PDGF expression was significantly increased in inflamed colonic mucosa but not in noninflamed mucosa; they suggest that PDGF may be important for the maintenance of damaged vasculature during IBD and an autocrine mechanism of inflammatory angiogenesis. Production of TGF-β has been examined in cells cultured from active CD and UC patients. Interestingly, in intestinal cells cultured from CD patients, less TGF-β was produced than in control cells, which is opposite in cells from UC patients (40). This indicates differential regulation of angiogenesis in CD and UC similar to what we have observed in experimental colitis models (31). HGF has also been shown to be upregulated during IBD. Interestingly, administration of human HGF and HGF gene therapy decrease disease severity in experimental colitis, an effect that is opposite of that of most growth factors during IBD (13, 62). These data combined with the VEGF data indicates growth factors as primary mediators of inflammation induced angiogenesis in experimental and human IBD.

Adhesion molecules and integrins. In addition to growth factors, cellular adhesion molecules (CAMs) and their ligands, selectins, and cadherins also have an extensive role in colitis-associated angiogenesis and are present at high levels during colitis. Adhesion molecules are important for endothelial cell-cell interactions and interactions with other cells such as leukocytes, monocytes, platelets, plasma cells, and ECM components. Intercellular adhesion molecule-1 (ICAM-1, CD54)/ β_2-integrins, vascular cell adhesion molecule-1 (VCAM-1)/ $\alpha_4\beta_1$ and β_2-integrins, platelet endothelial cell adhesion molecule-1 (PECAM-1, CD31)/$\alpha_v\beta_3$-integrin, mucosal addressin cellular adhesion molecule-1 (MadCAM-1)/$\alpha_4\beta_7$, CD146, P- and E-selectin, and VE-cadherin play important roles in recruiting cell types which produce angiogenic factors (Fig. 3) (4, 9, 12, 31, 41, 43). These molecules are expressed on the surface of vascular endothelium and are upregulated in response to angiogenesis or inflammation.

ICAM-1 and VCAM-1 are particularly important endothelial cell adhesion molecules that facilitate the adhesion and infiltration of leukocytes that produce mediators of angiogenesis at the inflammatory site. ICAM-1 is reported to be expressed at high levels in IBD patients and during experimental colitis (31, 160). Its ligands are members of the β_2 family of integrins (LFA-1, Mac-1, CD11c/CD18, CD11d/CD18) and interaction mediates firm adhesion of and extravasation of leukocytes. Interestingly, in the CD4$^+$CD45RB^{high} and DSS models, disease does not develop in LFA-1-null mice, indicating the importance of this molecule for leukocyte recruitment during experimental colitis (113). This corresponds with data from Bernstein et al. (16), who reported that there is a selective increase in lymphocytes expressing LFA-1 during CD and UC. We have also reported that loss of all β_2-integrins in DSS colitis results in severely decreased leukocyte recruitment and disease pathology with a concomitant reduction in vascular density accenting the role of increased leukocyte recruitment in pathological angiogenesis during experimental colitis (1, 31). Moreover, Bendjelloul et al. (14) found that ICAM-1$^{-/-}$ mice are protected in DSS-induced colitis, providing further evidence for the importance of this molecule in IBD. VCAM-1, which binds $\alpha_4\beta_1$ and members of the β_2 integrin family, is present on endothelium typically upon activation (expression induced by inflammatory cytokines) and is involved in the slow rolling of monocytes and lymphocytes during inflammatory cell recruitment. VCAM-1 has been indicated as an important molecule for the vascularization of tumors, through observation of microvascular density, protein expression, and VCAM-1 serum concentrations in cancer patients (43, 60). Additionally, VCAM-1 is essential for activation of B cells, which release many proangiogenic molecules (27).

PECAM-1, an adhesion molecule that is highly involved in angiogenesis, is abundantly expressed on endothelium (concentrated at intercellular junctions) and platelets, and to a lesser extent on leukocytes (41). PECAM-1 is indicated in facilitating leukocyte transmigration across endothelial cells in inflamed tissue (30, 116, 159). PECAM-1 interacts with itself in a homotypic manner and with $\alpha_v\beta_3$-integrin, whose expression is upregulated on activated endothelium during experimental colitis and in CD and UC (31, 37, 66). The involvement of PECAM-1 in angiogenesis was first demonstrated through PECAM-1 inhibition in rats by DeLisser et al. (41). Subsequent studies showed its involvement in tube formation and that

inhibition of PECAM-1 inhibits tumor angiogenesis (94, 169). During vascular remodeling $\alpha_v\beta_3$-integrin is associated with angiogenesis by stimulating endothelial cell proliferation and stabilizing endothelial-matrix interactions partially through its interaction with PECAM-1 (25, 93). PECAM-1 is also implicated in endothelial cell migration, specifically being shown to be required for elongation of endothelial cells and invasion into 3D collagen gels (74, 166). Importantly, PECAM-1 expression is not upregulated during inflammation; however, new vasculature constitutively expresses PECAM-1, making it an ideal marker for measuring changes in vascular density during IBD and experimental colitis (31, 162).

MadCAM-1 and CD146 may also take part in IBD-associated angiogenesis. MadCAM-1 is present in the mucosa of the gut on endothelial cells in the mesenteric lymph nodes and the lamina propria of the intestines and it binds $\alpha_4\beta_7$-integrin (4). MadCAM-1 is responsible for recruiting immune cells to the gut and is upregulated in experimental and active human IBD (23, 31). In fact, MadCAM-1 is considered the "gateway" recruiter of immune cells to the gut and its upregulation is thought to trigger inflammatory damage to the gut, which in turn causes angiogenesis (4). CD146 or melanoma cellular adhesion molecule was first discovered as a T cell antigen and is now known to be an endothelial cell junctional adhesion molecule similar to PECAM-1 (11, 117). This adhesion molecule is involved in later stages of metastasis and has been identified as an indicator of angiogenesis, but its specific biological role in angiogenesis is not well understood (53, 137). CD146 is reported to be upregulated in endothelial cells of CD and UC patients (12). High expression of these molecules in IBD may lead to increases in angiogenic factor-producing cell types, contributing to pathological angiogenesis during disease.

Lastly, VE-cadherin and selectins are additional adhesion molecules associated with angiogenesis in colitis. VE-cadherin is expressed on endothelial cells and is involved in angiogenesis primarily as a regulator of VEGF receptor function and vessel maturation (9). This molecule is an important stabilizing factor of blood vessels as it forms complexes with catenins on adjacent endothelial cells strengthening the junctions between adjacent cells. An important angiogenic role for VE-cadherin was identified by Yang et al. (166), who showed it is not only required for cell-cell adhesion during vessel development but is also involved in lumen formation through directing vacuole fusion (9, 166). Importantly, we observed VE-cadherin to be upregulated during CD4$^+$CD45RB^{high} colitis (31). Moreover, P- and E-selectin are rapidly mobilized in response to inflammation and act as capturing molecules to initiate leukocyte rolling on the endothelium (153). Studies in experimental colitis models have shown that inhibition of E- and P-selectin-mediated leukocyte rolling and adhesion results in attenuation of disease (129). Recruitment of inflammatory and immune cells during colitis is dependent on interactions between selectins and their ligands, which are upregulated during colitis, that may serve to perpetuate inflammatory angiogenesis.

The adhesion molecules listed above may also act in concert with growth factors to increase the presence of cell types capable of stimulating further angiogenesis. It is known that the angiogenic response is closely correlated to an increase in inflammatory cell types in the inflamed colon. Initial reports examining the relationship between inflammation and angio-

genesis showed that VEGF-A can facilitate monocyte chemotaxis and increase leukocyte adhesion (35, 42). A study by Detmar et al. (42) reported that transgenic overexpression of VEGF$_{164}$ in the skin of mice led to large increases in leukocyte rolling and firm adhesion and that these mice showed increased vascular density composed of immature vessels, indicating a molecular link between leukocyte recruitment and angiogenesis. Recent reports also demonstrate that VEGF-A is a potent immune modulator under several conditions such as ischemia-reperfusion and alloimmunity (126, 156). VEGF-A stimulation of human umbilical vein endothelial cells increases the expression of adhesion molecules, such as ICAM-1, VCAM-1, and selectins (68, 75). This induction of adhesion molecule expression increases in vitro leukocyte adhesion under static conditions, which occurs through the activation of VEGF-R2 (75, 76). Importantly, we have recently reported that VEGF-A stimulates increased ICAM-1 expression on distal colon microvascular endothelium, which facilitates neutrophil and T cell adhesion in a CD18-dependent manner (55). Our group has also shown that ICAM-1 influences control of VEGF-A induced endothelial NO synthase (eNOS) activity, endothelial chemotaxis, and angiogenesis through modulation of endothelial cell signaling pathways (84). Together, these data provide compelling evidence that angiogenic mediators can act in association with adhesion molecules to facilitate inflammation during IBD, which likely exacerbates disease through positive feedback pathways.

Matrix molecules. MMPs, specifically MMP-2 and -9, are elevated during experimental colitis and are involved in different aspects of angiogenesis (31, 36, 69). MMP-1 (interstitial collagenase), MMP-2 (gelatinase A), and MMP-9 (gelatinase B), produced by endothelial cells, are upregulated and have been shown to be crucial for tissue remodeling associated with angiogenesis. These MMPs are zinc-dependent enzymes, secreted as zymogens that are activated in the ECM that can be inhibited by tissue inhibitors of metalloproteinases (TIMPs) (Fig. 3) (115). These molecules dissolve fibrin in the basement membrane and have collagenolytic activity, which is important in the process of cell migration (69). Cornelius et al. (36) have indicated dual roles for MMPs in angiogenesis acting as proangiogenic mediators during tissue remodeling and then as antiangiogenic mediators through generation of angiostatin preventing vessel maturation. Additionally, overexpression of MMPs and TIMPS during UC has been shown by von Lampe et al. (163). Thus MMPs function in IBD angiogenesis may contribute to the pathology of disease by inhibition of vascular maturation while promoting early vessel growth.

Signaling targets. Several other factors involved in regulating angiogenesis include caveolin-1 and -2 (Cav-1 and -2), NO, and endoglin-1 (Edg-1). Cav-1 and Cav-2 are major structural components of caveolae that form a signaling platform at the surface of endothelial cells. Importantly, Cav-2 upregulation has been observed clinically in UC and is highly involved with other angiogenic mediators shown to be involved in IBD (5). It is also thought that enhanced Cav-2 expression alters signal transduction in UC, thus indicating a role for caveolae in IBD (5). Cav-1 is associated with VCAM-1 in progression of tumor vasculature and is necessary for remodeling the vasculature (138, 167). Additionally, Sonveaux et al. (142) showed that Cav1$^{-/-}$ mouse cells exhibit poor tube formation in cultured endothelium (32). Cav-1 expression has been shown to prevent

NO production in response to VEGF-A activation of VEGFR2 by acting as a competitive inhibitor of eNOS (24, 63). This is important for pathological angiogenesis, because NO has an inhibitory effect on leukocyte recruitment through downregulation of P-selectin, as a mechanism of decreasing inflammatory responses (2). Importantly, VEGFR2 and eNOS colocalize in caveolae, and this is the site of VEGF-A stimulated NO production (20). This is important for angiogenic activity because NO is a critical signaling molecule necessary for endothelial cell differentiation and motility. Also, Feng et al. (45) have shown that endothelial cell permeability mediated by VEGF-A is mediated by caveolae. These data suggest that caveolae may be important for pathological angiogenesis during experimental colitis.

NO is a major endothelium-derived relaxing factor, thus an important regulator of blood flow. In addition, it increases production of VEGF-A during inflammation and mediates VEGF induced angiogenesis during its early stages (52, 102). Two forms of NO synthase are involved in NO production during IBD and experimental colitis: eNOS and inducible NO synthase (iNOS). eNOS is a constitutive source of NO whereas iNOS is an inducible generator of NO during disease pathology (103, 108, 109). Genetic deletion of eNOS expression in the endothelium in the DSS and TNBS models resulted in increased disease activity, suggesting a protective role for eNOS derived NO acting in antioxidant, anti-inflammatory roles (132, 133, 161). Krieglstein et al. (80, 81) have also shown that loss of iNOS is protective in DSS colitis; they suggest that iNOS-derived NO may be pathogenic and that tissue-derived iNOS makes a larger contribution to inflammatory cell recruitment than blood cell-derived iNOS. These data show that control of NO production is altered during disease and plays a role in differential immune regulation.

Additionally, Edg-1 is a G-protein coupled receptor for sphingosine-1-phosphate (S1P) that is induced during endothelial cell differentiation and upregulated in experimental colitis (31). Edg-1 initiates S1P signaling involved in cell proliferation, survival, migration, morphogenesis, adhesion molecule expression, and cytoskeletal changes (34, 56, 61, 97, 144). Thus the combined effects of these signaling molecules in a disease state may exacerbate the underlying pathology of IBD, leading to increases in vascularity.

Inflammatory mediators. It is now appreciated that classical inflammatory cytokines and molecules such as TNF-α and IL-1β can stimulate both endothelial inflammatory and angiogenic pathways. However, it has recently been shown that the proangiogenic factors angiopoietin-2 and VEGF-A can facilitate increased inflammatory endothelial cell activation. A recent study by Fiedler et al. have clearly shown that angiopoietin-2 expression sensitizes endothelial cells to TNF-α activation and adhesion molecule induction (48). Studies also demonstrate that VEGF-A directly affects T cell responses. A study from Mor et al. (98) reported that activated T cells produce and release VEGF-A that can then stimulate naive T cells toward a Th1 phenotype. This evidence highlights the notion of a reciprocal relationship between inflammation and angiogenesis during colitis and supports the concept of pathological angiogenesis in the sustenance and maintenance of disease.

Several cytokines and chemokines including TNF-α, IL-1β, IL-8, IL-10, IL-12, IL-18, MCP-1, and Gro-1 are known to be

proinflammatory but may also be involved in angiogenesis during IBD (64, 89, 118, 145, 152). TNF-α, a potent inflammatory cytokine known to modulate angiogenesis, is upregulated in the inflammatory response, secreted by monocytes, fibroblasts, and smooth muscle cells, activating endothelial cells causing the release of inflammatory and angiogenic mediators. Importantly, the majority of TNF-α's proangiogenic effects occur in response to its proinflammatory actions, as discussed under *Relationship Between Inflammation and Angiogenesis*. Coinciding with this, TNF-α can upregulate expression of VEGF-R2 and NP1, the ligands for VEGF$_{165}$, leading to promotion of angiogenesis (54). In a study of UC and CD patients TNF-α receptor levels were found to be elevated during both active and remission phases of disease, suggesting the possibility that TNF-α receptors may mediate angiogenic activity in IBD (31, 145). Importantly, TNF-α receptor activation can facilitate transactivation of VEGFR2 through the Etk pathway, resulting in mimicked VEGF-A angiogenic simulation (110, 168). Additionally, CD40, a member of the TNF receptor gene family, and CD40 ligand (CD40L or CD154) interactions are multifunctional effectors of the immune response that cause upregulation of adhesion molecules on endothelial cells and increased expression of cytokines and chemokines by immune cells. CD40 and CD40L are upregulated during IBD and have been shown to promote endothelial cell migration indirectly through proangiogenic cytokine release in vitro (39). Importantly, CD40 and CD40L knockout mice show protection from inflammation and reduced angiogenic response during DSS colitis (39).

The proinflammatory cytokine IL-8 is also proangiogenic, upregulated in response to hypoxia, and increased during experimental colitis (31). Tissue levels of IL-8 have been reported to be upregulated in cases of CD and UC (37). IL-1β is crucial to IBD pathology, and its inhibition has been shown to attenuate DSS colitis through inhibition of proinflammatory activity and possibly downstream proangiogenic activity (91). IL-12 and IL-18 are inducers of IFN-γ and TNF-α production in lymphocytes; however, observations in DSS colitis revealed that IL-12$^{-/-}$ mice develop mild colitis whereas IL-18$^{-/-}$ mice develop severe colitis, indicating that IL-12 may be more protective than IL-18 (152). IL-12 has been shown to be required in the angiostatin cascade, suggesting that it may downregulate pathological angiogenesis (105). IL-18 is also known to be produced by macrophages in CD as well as being a mediator of tube formation in Matrigel plugs in mice with rheumatoid arthritis, implicating it in angiogenesis (64, 112). Additionally, IL-12 and IL-18 have been shown to play a role in increased expression of Th1 cytokines in CD (90, 135). IL-10 inhibits antigen presentation and thus inflammatory cytokine release during IBD. This is consistent with the fact that IL-10$^{-/-}$ mice spontaneously develop colitis (82). IL-10 is overexpressed in patients with active UC, and IL-10 therapy has had success in experimental colitis and IBD treatment (7, 96, 134). This could be due to the fact that IL-10 stimulates TIMP-1 production as well as reducing secretion of MMP-2 and -9 in in vitro assays, preventing ECM degradation (146). Together TNF-α and the interleukins offer a large contribution to the regulation of angiogenesis as well as inflammation during experimental colitis and IBD.

MCP-1 is an inflammatory chemokine, upregulated during experimental colitis and IBD, that can be induced by TNF-α and can mediate increases in vascular permeability through reducing endothelial tight junctions (77, 87). MCP-1 can also induce monocyte-endothelial cell interaction and transmigration, thus recruiting angiogenic cytokines to the inflammatory site (118). Gro-1 is an autocrine growth factor and a chemokine also upregulated in experimental colitis (31). Immunohistochemical staining of tumors indicates that Gro-1 colocalizes with VEGF during cancer development and its expression is upregulated in association with increased microvascular density (89, 139). The effects of these upregulated chemokines are likely to contribute to pathological angiogenesis in IBD through facilitating leukocyte recruitment to inflamed tissue or by directly activating endothelium.

Antiangiogenic Gene Expression and Therapeutics in IBD and Experimental Colitis

The involvement of antiangiogenic factors in experimental colitis and IBD is an important part of the differential regulation observed in IBD. Angiostatin is known to be antiangiogenic and involved in mediating vascularization during colitis as discussed under *Inflammatory Mediators*. Additionally, endostatin has been observed to be upregulated in TNBS colitis and in UC (131). Endostatin is a proteolytic fragment of collagen type XVIII and is shown to inhibit angiogenesis through antimigratory and antiproliferative mechanisms (165). In the TNBS model this upregulation occurs at 6 h; however, in the DSS model endostatin and angiostatin are not upregulated upon completion of the 6-day model, suggesting a temporal response for these molecules (31, 131). The upregulation of these and other antiangiogenic mediators underscores the complexity of the differential angiogenic response during experimental colitis and IBD and warrants further investigation to determine its relationship.

The first concepts of antiangiogenic drugs were introduced for cancer treatment. However, with our increased understanding of the involvement of angiogenesis during inflammatory diseases, these drugs can be expanded for use in many pathological conditions. Recent studies have investigated antiangiogenic treatment of experimental colitis models and IBD patients in an effort to attenuate disease. Several antiangiogenic compounds have been successful in inhibiting large increases in vascular density while reducing the overall pathology of disease. In a clinical report in 1999, a patient with CD was treated with the antiangiogenic agent thalidomide; the result was complete remission of disease over a 4-year period while on thalidomide therapy (51). Our group and others have also shown that thalidomide significantly attenuates disease histopathology and increases in vascular density in the DSS model of experimental colitis (31, 37).

Other antiangiogenic therapies are currently being tested in experimental colitis and IBD. We have used a novel antiangiogenic agent, ATN-161, in the CD4$^+$CD45RB^{high} model and observed decreases in tissue histopathology and blood vessel density (31). ATN-161 is a specific $\alpha_v\beta_3$, $\alpha_5\beta_1$-integrin blocker shown to antagonize tumor angiogenesis and endothelial cell growth in Matrigel. Also, a recent study by Danese et al. (37) examined the effects of ATN-161 in the IL-10 model and they also reported decreased disease activity and significantly lower histopathology scores and blood vessel density vs. control animals. Anti-$\alpha_v\beta_3$-integrin therapy has

also been examined by use of Vitaxin, an anti-$\alpha_v\beta_3$-specific antibody, on mucosal endothelial monolayers; this study reported induction of apoptosis in activated endothelium with increased $\alpha_v\beta_3$ expression, theoretically preventing angiogenesis by preventing vessel sprouting (37). Additionally, anti-TNF-α treatment, although technically anti-inflammatory, also inhibits the initiation of angiogenesis as discussed in this review. Data show that infliximab, a monoclonal anti-TNF-α antibody, has had promising results in treatment of patients with CD (147). Additional biological therapies, antibodies against other angiogenic cytokines (such as IL-10, IL-12, and IFN-γ), are also being considered as therapeutic targets in IBD (7, 114). Thus antiangiogenic therapies have the potential to revolutionize the way we treat IBD.

The conceptual findings discussed above indicate that IBD pathogenesis may be characterized by differential angiogenic regulation contributing to and perpetuating a chronic inflammatory state in the bowel. Augmentation of antiangiogenic gene expression or neutralization of proangiogenic mediators appears to be an ideal future path for the treatment of IBD. The preliminary success seen in clinical settings with the antiangiogenic agent thalidomide and the significant amount of progress being made with experimental antiangiogenic therapies indicates a new and effective therapeutic avenue. Greater understanding of how pathological angiogenesis is differentially regulated during colitis is clearly needed, but the findings discussed here show that we are making progress.

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