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Review

Multiple Pathogenic Roles of Microvasculature in Inflammatory Bowel Disease: A Jack of All Trades

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The etiology of Crohn's disease and ulcerative colitis, the two major forms of inflammatory bowel disease (IBD), is still largely unknown. However, it is now clear that the abnormalities underlying pathogenesis of intestinal inflammation are not restricted to those mediated by classic immune cells but also involve nonimmune cells. In particular, advances in vascular biology have outlined a central and multifaceted pathogenic role for the microcirculation in the initiation and perpetuation of IBD. The microcirculation and its endothelial lining play a crucial role in mucosal immune homeostasis through tight regulation of the nature and magnitude of leukocyte migration from the intravascular to the interstitial space. Chronically inflamed IBD microvessels display significant alterations in microvascular physiology and function compared with vessels from healthy and uninvolved IBD intestine. The investigation into human IBD has demonstrated how endothelial activation present in chronically inflamed IBD microvessels results in a functional phenotype that also includes leakiness, chemokine and cytokine expression, procoagulant activity, and angiogenesis. This review contemplates the newly uncovered contribution of intestinal microcirculation to pathogenesis and maintenance of chronic intestinal inflammation. In particular, we assess the multiple roles of the microvascular endothelium in innate immunity, leukocyte recruitment, coagulation and perfusion, and immune-driven angiogenesis in IBD. ***(Am J Pathol 2008, 172:1457–1466; DOI: 10.2353/ajpath.2008.070593)***

The two major forms of inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC), represent classic chronic inflammatory disorders characterized by progressive destructive inflammation in the gastrointestinal tract. Although their etiology is still unknown, major progress has occurred in our understanding of the pathogenic mechanisms underlying intestinal inflammation.[1] In particular, the importance of vascular involvement in IBD has been recognized over the past four decades.[2] It is now clear that the abnormalities underlying IBD pathogenesis are not restricted to those mediated by classic immune cells, such as T and B lymphocytes, macrophages, and dendritic cells, but also involve nonimmune cells.[3] Advances in vascular biology have delineated a central role for the microcirculation in the initiation and perpetuation of the inflammatory process (Table 1).

The endothelium is a highly specialized cellular system that performs numerous and varied biological tasks and plays a crucial role in multiple physiological processes, such as flow of nutrients, blood flow, tissue homeostasis, and cell trafficking and distribution, as well as pathological processes such as inflammation. Endothelial cells (ECs) play a key role in mucosal immune homeostasis by regulating the quality (type) and quantity (number) of leukocytes migrating from the intravascular to the interstitial space, thus highlighting the endothelium as one of the pillars in inflammation pathogenesis.[4,5] Indeed, the vascular response is a key component of inflammation, whereby tissue ECs become activated and display a functional phenotype including leakiness, leukocyte adhesiveness, procoagulant activity, and eventually angiogenesis.[6] During inflammation, the mucosal microvasculature controls the nature and magnitude of leukocyte influx through cell adhesion molecule (CAM) expression and chemokine secretion, which further amplify the communication with leukocytes and other cells.[5] Microvascular ECs have the potential to form capillary-like structures and display different functional sets of adhesion mole-

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Table 1. Endothelial Responses and Their Histopathological/Clinical Manifestations

Endothelial activation and expression of adhesion molecules	↑ leukocyte adhesion and transmigration ↑ platelet adhesion
EC tissue factor expression	Hypercoaguability Fibrotic occlusions
↑ vascular permeability Vascular leakiness	Edema
Angiogenesis	Remodeling of the vascular bed ↑ influx of inflammatory cells
Vasodilatory microvascular dysfunction	Hypoperfusion/ischemia Granulomatous vasculitis Ulceration/tissue necrosis

cules, distinct chemokine secretory patterns, and activation of unique sets of genes in response to stress and inflammatory stimuli.[7,8] Even though many insights into the function of ECs have been gained through studies of cultured human umbilical vein endothelial cells (HUVECs), it is evident that HUVECs cannot substitute for specialized tissue microvascular ECs. An important turning point in the definition of the contribution of the microvascular endothelium in chronic intestinal inflammation was the development by Binion and colleagues[8] of protocols for routine isolation and long-term culture of pure populations of human intestinal microvascular endothelial cells (HIMECs), which demonstrate unique patterns of leukocyte adhesion and growth compared with HUVECs. It is believed that local concentration of tissue-specific mediators and transcription factors contribute to the induction or the maintenance of a specific tissue EC profile.[5,8–10]

This review assesses the role of the microvasculature in IBD and its newly uncovered contribution to pathogenesis and maintenance of intestinal inflammation. In particular, we review the role of the microvascular endothelium in innate immunity, leukocyte recruitment, coagulation and perfusion, and immune-driven angiogenesis.

Microvascular Endothelium and Leukocyte Recruitment in IBD

Endothelial activation in response to cytokines and inflammatory mediators leads to leukocyte recruitment from the circulation, where CAMs and chemokines expressed by ECs mediate enhanced leukocyte interaction, as well as the multistep extravasation cascade, which includes tethering/rolling, activation, adhesion, spreading, and transmigration (Figure 1, box 1).

Initial studies of endothelial contribution to IBD inflammation focused on the microvascular expression of CAMs, which mediate recruitment of circulating leukocytes.[11,12] Briskin and colleagues[13] demonstrated an increase in the gut-specific homing molecule mucosal addressin cell adhesion molecule-1 (MAdCAM-1), which plays a major role in the recruitment of α4 integrin-expressing leukocytes into the mucosal immune compartment. Salmi and colleagues[14] investigated the potential alterations in leukocyte homing patterns in IBD using an *ex vivo* leukocyte-binding assay on thin sections of control and IBD bowel. They demonstrated that naïve lymphocytes are preferentially recruited to IBD intestinal microvascular endothelium, compared with control microvessels that bind increased numbers of memory lymphocytes. These findings were confirmed by Burgio and colleagues,[15] who also demonstrated an altered pattern of leukocyte binding in CD, where naïve T cells and monocytes were again preferentially recruited to the chronically inflamed intestine.

Subsequent studies performed with HIMECs isolated from both chronically inflamed CD and UC intestines demonstrated a significantly enhanced capacity of these cells to adhere leukocytes compared with control HIMECs. This outcome was only elicited after activation with proinflammatory cytokines [interleukin (IL)-1β and tumor necrosis factor α (TNF-α), but not IL-4] and bacterial lipopolysaccharide (LPS) but was not present in unstimulated cells. Furthermore, enhanced leukocyte adhesion was only present in HIMECs from chronically inflamed areas, as cultures derived from uninvolved areas in close proximity do not exhibit increased leukocyte binding.[16] The established role of nitric oxide (NO) in IBD[17] compelled the investigation of mechanisms underlying leukocyte hyperadhesion to focus on NO generation in HIMECs, an alternate pathway that would influence the activation status of these tissue-specific ECs, and their capacity to bind circulating leukocytes.[18] Control HIMECs displayed distinct patterns of NO generation through both constitutive endothelial NO synthase (eNOS or NOS3) as well as inducible NO synthase (iNOS or NOS2). In marked contrast, IBD-derived HIMECs demonstrated a loss of iNOS gene expression after activation that corresponded to a decrease in NO generation and enhanced leukocyte binding.[19,20] Recent evidence also suggests that increased arginase activity in HIMECs exposed to an inflammatory milieu contributes to the loss of NO production.[21]

Apart from classic adhesion molecules, the unique CX_3C chemokine fractalkine (FKN), which acts as an adhesion molecule, has been shown to be up-regulated in the endothelium of both CD and UC patients.[22] Indeed, HIMECs isolated from IBD patients display an increased expression of FKN after stimulation with the combination of interferon γ (IFN-γ) and TNF-α compared with HIMECs derived from control subjects. Moreover, greater numbers of T cells expressing CX_3CR1 are found in the circulation of IBD patients than in healthy subjects.[23] These observations indicate FKN as an important mediator of endothelium-leukocyte interaction in intestinal inflammation, suggesting a role for EC-derived FKN in the induction of a localized proinflammatory response through multiple complementary functions that include leukocyte retention, integrin affinity up-regulation, chemoattraction, and transmigration.

Recently, homocysteine was also shown to participate in microvascular inflammation in IBD. The treatment of gut-derived ECs with homocysteine, or with a combination of TNF-α and homocysteine (which act synergistically), triggers endothelial activation, resulting in vascular cell adhesion molecule-1 (VCAM-1) up-regulation, monocyte chemotactic protein-1 (MCP-1) production, and mitogen-activated protein kinase (MAPK) p38 phosphory-

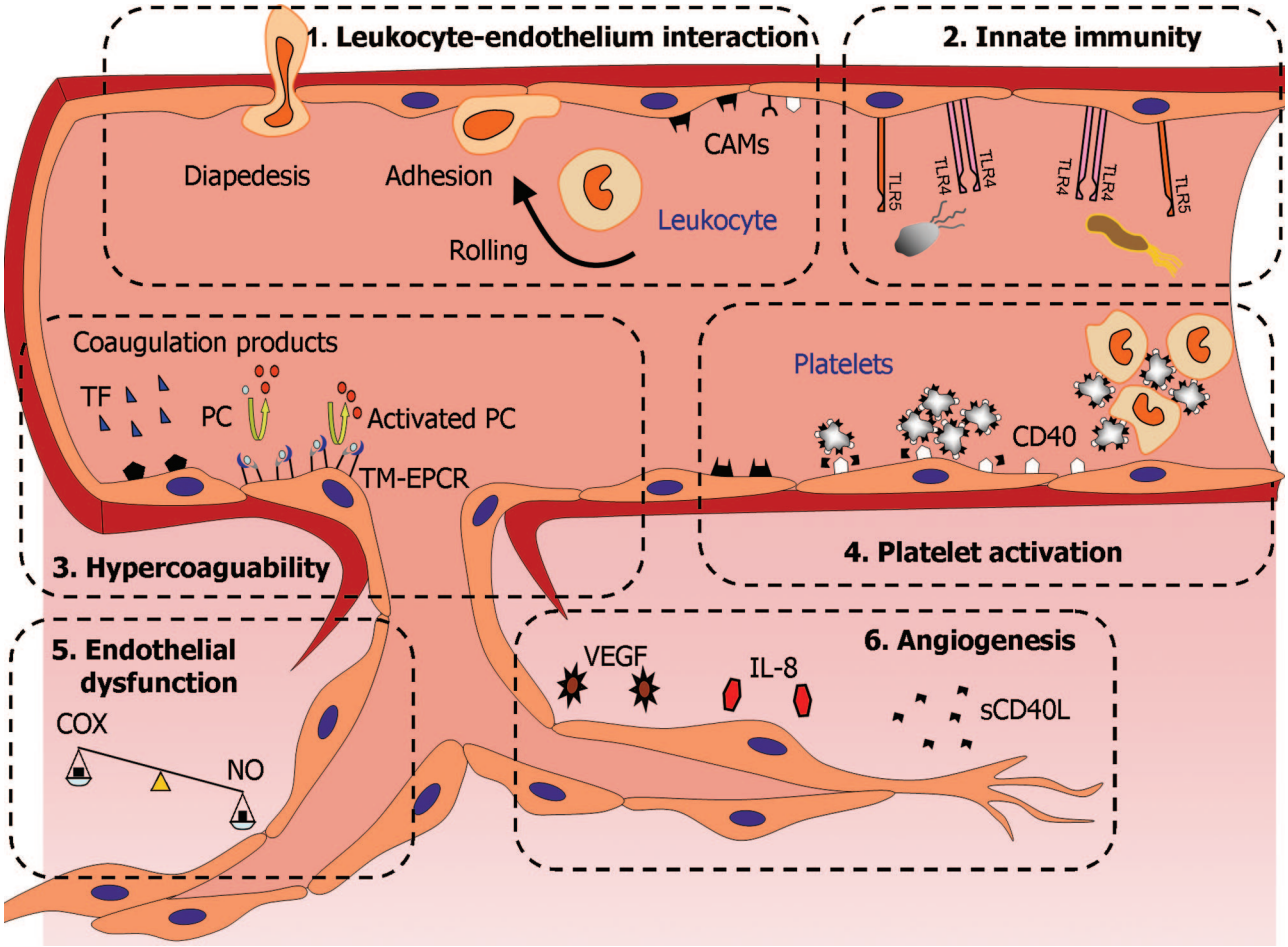

Figure 1. The role of microvasculature in the pathogenesis of inflammatory bowel disease. **1. Leukocyte-endothelium interaction:** The chronically inflamed endothelium displays enhanced leukocyte binding capacity and up-regulation of adhesion molecules. **2. Innate immunity:** Pattern recognition receptors contribute to the activation of intestinal endothelium, increasing leukocyte adhesion and transmigration and contributing to the cytokine network of the gut mucosa. **3. Hypercoaguability:** In IBD, a hypercoagulable state and a prothrombotic condition exist; in particular, the impairment of expression and function of the PC pathway and the overexpression of tissue factor suggest the biological importance of coagulation cascade and its alterations in intestinal inflammation. **4. Platelet activation:** The intimate adherence of platelets to the endothelium is characteristic of IBD, and it is well established that platelets behave aberrantly in both UC and CD. **5. Endothelial dysfunction:** Intrinsic alterations in the chronically inflamed and remodeled microcirculation underlie vascular dysfunction that seems to play a fundamental role in chronic, deregulated inflammation, which characterizes IBD. **6. Angiogenesis:** The enhancement of angiogenesis in IBD highlights neovascularization as a major contributor to the initiation and perpetuation of chronic intestinal inflammation.

lation. These events lead to an increased capacity of the endothelium to adhere T cells and monocytes, indicating a proinflammatory role of homocysteine in IBD.[24] Additional insight into inflammatory pathways involved in IBD have emerged from a recent study by Vowinkel et al.[25] The authors report a great increase in endothelial expression of CD40 and reveal the critical role of the CD40-CD40L signaling pathway in the leukocyte recruitment to inflamed murine colon.

The enhanced leukocyte binding capacity and up-regulation of adhesion molecules by chronically inflamed endothelium reveal the central role of microcirculation in the establishment of the cycle of inflammation in IBD and underline the complex pathogenesis of these conditions.

Intestinal Microcirculation and Innate Immunity

The function of innate immunity is the elimination of invading microbes from the gastrointestinal tract and the control of their systemic dissemination and, as such, represents a critical first line of defense. Recognition is mediated by pattern recognition receptors (PPRs) that distinguish self from conserved structures shared by different microorganisms.[26] Among such receptors, Toll-like receptors (TLRs) are molecules essential for microbial binding, and play a central role in the initiation of innate cellular immune responses and the subsequent adaptive immune responses to microbial pathogens. ECs express several TLRs, and their signaling leads to the induction of numerous genes that function in host defense, including those for inflammatory cytokines, chemokines, antigen-presenting molecules, and costimulatory molecules[26] (Figure 1, box 2). Thus far, the expression of TLR4 and TLR5 has been reported on intestinal microvascular ECs.

TLR4 functions as a sensor for LPS, part of the cell membrane of Gram-negative bacteria, and as such has been considered a strong functional candidate in the pathogenesis of IBD as well as in other immuno-inflam-

matory diseases.[27] Haraldsen and colleagues[28] found that E-selectin, VCAM-1, and intercellular adhesion molecule-1 (ICAM-1) could be induced or up-regulated in a dose-dependent fashion on HIMECs by LPS. However, unlike in HUVECs, LPS stimulation did not maintain prolonged expression of ICAM-1 and VCAM-1 in HIMECs.[28] Accordingly, Ogawa and colleagues[29] showed significantly decreased leukocyte binding in HIMECs undergoing prolonged, repeated activation in response to LPS. This regulated physiological response to repeated LPS challenge is mediated in part through inhibition of MAPK signaling cascades, altered cytokine and CAM expression, enhanced oxy radical defense via increased expression of manganese superoxide dismutase (Mn-SOD), and diminished intracellular superoxide anion. Because the binding of circulating leukocytes to the microvascular endothelium is the initial event in leukocyte extravasation and tissue inflammation, the attenuated binding activity in HIMECs after repeated LPS exposure may represent an important down-regulatory mechanism to prevent excessive inflammation in response to chronic LPS exposure and to promote intestinal tolerance.[29]

TLR5 is the Toll-like molecule that recognizes flagellin from both Gram-positive and Gram-negative bacteria. Activation of this receptor mobilizes NF-κB and stimulates TNF-α production.[30] Because infection of intestinal epithelial cells with *Salmonella* leads to an active transport of flagellin to the subepithelial compartment in proximity to microvessels, Maaser and colleagues[31] specifically studied TLR5 expression and function in HIMECs, HUVECs, and dermal ECs. The authors showed that *Salmonella*-infected intestinal epithelial cells induced ICAM-1 expression in co-cultured ECs. All three types of ECs constitutively expressed high levels of TLR5 mRNA and protein. The functional role of endothelial TLR5 was demonstrated by induction of leukocyte adhesion and transmigration, pointing to a previously unrecognized role of endothelial TLR5 in the innate immunity.[31]

Nielsen and colleagues,[32] who studied the cytokine expression profile of HIMECs by exposing cultured cells to recombinant cytokines and LPS, provide additional evidence for the involvement of microcirculation in innate immunity. Their work indicates that intestinal microvascular endothelium can constitutively produce several cytokines, which are up-regulated after stimulation with cytokines or LPS, suggesting that microvasculature may contribute to the cytokine network of the gut mucosa with the ability to respond to locally generated cytokines and to produce potent inflammatory mediators.

Platelet-Endothelial Interactions

The intimate adherence of platelets to the endothelium is a general phenomenon characteristic of the early manifestations of regional immune reactivity[33] and persists throughout the course of several inflammatory conditions, including IBD.[34] It is now well established that platelets behave aberrantly in both CD and UC.[35] Observation of increased platelet aggregates in the mesenteric blood of CD patients suggested platelet activation in IBD mucosa[36–38] (Figure 1, box 4). This phenomenon was recently reproduced *in vitro* using platelets co-cultured with HIMECs. Pretreatment of HIMECs with IL-1β to mimic IBD endothelium can activate platelets through simple physical contact, as evidenced by a sustained up-regulation of P-selectin and CD40L expression on the platelet surface.[39]

Additional evidence of platelet involvement in mucosal inflammation is the recent demonstration that IBD platelets express high levels of surface CD40L, creating a physical and biological bridge that allows interaction with and activation of HIMECs. This series of events actually occurs, as CD40L-positive platelets in IBD have been detected *in vivo* adhering to mucosal microvascular endothelium, where they trigger or amplify a proinflammatory response.[40] The *in vitro* counterpart for this finding is the up-regulation of VCAM-1 and ICAM-1 by activated IBD platelets through the CD40-dependent pathway. Through this same pathway, IBD-derived platelets also stimulate HIMECs to produce IL-8, the major neutrophil chemoattractant, setting in motion the signaling machinery of HIMECs through the MAPK cascade, and promoting a marked phosphorylation of p38. It is worthy of note that platelets can activate various cells not only through contact with membrane-bound CD40L, but also through the release of its soluble form (sCD40L),[40] representing yet another paracrine mechanism of inflammation. For instance, sCD40L can activate intestinal resident cells such as fibroblasts and HIMECs, inducing them to secrete chemokines, up-regulate VCAM-1 and ICAM-1, and enhance T-cell adhesion to endothelium and subsequent transmigration into the interstitium.

In addition to IL-8, IBD platelets release, on contact with HIMECs, profuse amounts of biologically active RANTES (regulated on activation, normal T cell expressed and secreted),[41] a chemokine critical for recruitment of monocytes and memory T cells.[42] HIMECs avidly immobilize and retain on their surface the platelet-derived RANTES, which can thus mediate adhesion of more T cells to HIMECs. This sequence of events probably renders the unfolding of an *in vivo* inflammatory cycle, whereby platelet-triggered, chemokine-mediated leukocyte adhesion to endothelium occurs and subsequently results in leukocyte transmigration into the interstitium to create a focus of inflammation. This cycle links platelet activation and leukocyte recruitment and implicates platelets in cell-mediated immune phenomena in gut inflammation.[40]

Furthermore, leukocytes adhering to an inflamed microvascular bed may create an effective platform onto which platelets bind and further interact with the endothelium itself.[43] In particular, using an experimental colitis model, Vowinkel and colleagues[44] have recently shown that platelet-leukocyte interactions are mediated by CD40L, as revealed by the significant reduction of circulating platelet-leukocyte aggregates if CD40L-deficient animals are used. The following study showed that CD40 expression in the colonic vasculature was greatly elevated during dextran sodium sulfate (DSS)-induced inflammation in wild-type mice. Reduction in the recruitment of adherent leukocytes and platelets and

attenuation of disease activity after DSS administration was observed in both CD40L- and CD40-deficient mice and in wild-type animals treated with the CD40-CD40L pathway inhibitor Trapidil (triazolopyrimidine).[25] In addition, platelets and leukocyte aggregates are more frequent in IBD patients compared with healthy controls, and such aggregates are more likely to adhere to mucosal endothelium than are leukocytes that circulate alone and to induce up-regulation of the pro-coagulant molecule tissue factor (TF) by monocytes.[45] Mori and colleagues[46] have shown that the accumulation of adherent platelets in venules in the DSS-induced colitis model is temporally correlated with the accretion of adherent leukocytes and with disease severity, and that the recruitment of both platelets and leukocytes is largely mediated by P-selectin-P-selectin glycoprotein ligand-1 (PSGL-1) interactions. This study suggests that the recruitment of platelets and leukocytes on the colonic endothelium may be an interdependent process. Recent data by Vowinkel and colleagues[47] provide evidence in favor of such a supposition. The authors show that platelets exert a profound influence on the recruitment of leukocytes in DSS-induced colitis and that the number of circulating neutrophils also influences the nature and magnitude of platelet adhesion to endothelial cells. In particular, the overwhelming majority of platelets accumulate on the venular wall by attaching to rolling or firmly adherent leukocytes, with a smaller percentage of platelets binding directly to venular endothelium, and the presence of rolling and firmly adherent platelet-leukocyte aggregates in colonic venules during DSS colitis was profoundly reduced in mice rendered neutropenic, suggesting that neutrophils are the dominant leukocyte population binding to platelets in this model. Furthermore, the authors describe a dramatic inhibitory effect of thrombocytopenia on the rolling and firm adherence of leukocytes in inflamed colonic venules. This observation suggests that platelets play a major role in inducing the inflammatory phenotype that is assumed by leukocytes and/or venular endothelial cells during DSS colitis. This possibility is consistent with a report describing enhanced superoxide production by isolated neutrophils that are incubated with platelets isolated from patients with ulcerative colitis.[48] These recent observations underline the interaction between P-selectin, expressed on endothelial cells and platelets, with PSGL-1, expressed on the surface of leukocytes and endothelial cells as the major players in the accumulation of both cell populations in colonic vessels. However, it remains unclear whether platelets contribute equally in experimental IBD models that are more T-cell dependent.

Hypercoagulability and Prothrombotic State in IBD

Clinical experience and bench research have clearly demonstrated that, in both forms of IBD, a hypercoagulable state and a prothrombotic condition exist, whereas coagulation abnormalities are an intimate part of the IBD clinical picture.[49] In fact, thromboembolic disease is a significant cause of morbidity and mortality in patients with IBD.[50]

Hypercoagulability identifies the imbalance of the coagulation cascade toward the procoagulant forces due to an excessive activation of coagulation enzymes without clinical signs of thrombosis. Several studies have been published describing the markers of activation of coagulation, such as prothrombin fragment 1 + 2 (F1 + 2), the thrombin-antithrombin III complex (TAT), fibrinopeptide A (FPA), and fibrinopeptide B (FPB), indicating subclinical activation of coagulation in IBD.[39,51,52] It is debated whether this evidence of coagulatory cascade activation is secondary to chronic inflammation or represents a primary feature of IBD, independent of disease clinical activity. The potential protective effect of an underlying bleeding predisposition in preventing the development of IBD was investigated by Thompson and colleagues,[53] who found a significantly decreased risk of development of either CD or UC among patients with either hemophilia or von Willebrand's disease. The authors concluded that a congenital bleeding predisposition exerted a protective effect against development of IBD, suggesting an important role of inappropriate coagulation and vascular occlusion in the pathogenesis of human IBD.[53]

In IBD, increased plasmatic levels of several recognized risk factors for thrombosis, such as increased levels of factors V, VII, and VIII[54], lipoprotein (a)[55], and fibrinogen,[54] as well as reduced fibrinolytic activity, have been consistently described, indicating a prothrombotic condition.

In addition to the demonstration that coagulation abnormalities and thromboembolic complications are clinically relevant events in IBD, they have been shown to exert effects at the mucosal level, where a coagulative imbalance exists. In fact, one of the earliest abnormalities in CD mucosa is the presence of platelet thrombi cross-linked with fibrin in the mucosal microvasculature.[56] Indeed, crucial changes to the mucosal microvasculature comprising vascular injury, focal arteritis, fibrin deposition, microinfarction, and neoangiogenesis have been observed in CD,[53] as well as intracapillary clots in rectal biopsies of UC patients.[34] Moreover, endothelial injury and disruption could lead to exposure of the subendothelial matrix, to which platelets are strongly attracted, thus further promoting microthrombi formation.

The imbalance of the coagulant potential of the inflamed mucosal microvasculature in active IBD is further reflected by increased expression of TF, which closely correlates with the degree of thrombosis in the mucosal microvasculature of CD patients.[56] Accordingly, mouse TF-blocking antibody treatment prevented elevation in TAT complexes, reduced leukocyte and platelet recruitment and tissue injury, and blunted thrombus formation in DSS colitic mice. These findings further implicate TF in intestinal inflammation and support an interaction between inflammation and coagulation in experimental colitis.[57] On the contrary, a dramatic down-regulation in the expression of the anticoagulant thrombomodulin (TM) and endothelial protein C receptor (EPCR) has been reported in IBD microvessels[58] (Figure 1, box 3). A recent study by Scaldaferri and colleagues[59] demonstrates that

both TNF-α and IL-1β trigger prompt surface down-regulation of TM and EPCR, thus highlighting the crucial role of inflammatory mediators in governing their surface expression. The authors demonstrate that in resting conditions, HIMECs are able to convert protein C (PC) into its activated form, but that such a process is strongly inhibited after stimulation with proinflammatory cytokines, consistent with the down-regulation of TM and EPCR and reinforcing the notion that inflammation impairs PC conversion. Furthermore, *in vitro*, activated PC acts as a potent anti-inflammatory drug since it inhibited TNF-α-induced CAM up-regulation and chemokine secretion. The functional significance of this inhibition is confirmed by administration of activated PC in experimental models of colitis, where it inhibited leukocyte adhesion to inflamed intestinal microcirculation.[59] These *in vivo* and *in vitro* data suggest an impairment of the expression and function of the PC pathway in the IBD mucosal microcirculation and represent a proof-of-concept for the biological importance of the coagulation cascade and its alterations in intestinal inflammation.

Microvascular Alterations and Dysfunction in IBD

The growing evidence of the importance of the vascular system in intestinal physiology and homeostasis has prompted investigation into potential vascular alterations in IBD.[60] In the early stages of IBD, angiographic studies show arteries that abruptly decrease as the vessels reach the bowel wall with right-angle bifurcation, bizarre distribution, and small luminal irregularities in the peripheral branches.[61,62] In contrast, advanced IBD lesions have demonstrated reduced vessel diameter,[63] decreased vascular density, and diminished blood flow.[64]

Distinct patterns of vascular perfusion have been correlated with discrete phases of both CD and UC. Early colitis with severe inflammation is characterized by increased vascular perfusion, whereas, paradoxically, reduced regional blood flow is typically seen in chronically inflamed and remodeled tissues.[65–67] These early observations have been confirmed with endoscopic Doppler ultrasonography, demonstrating a diminished vascular perfusion associated with fibrosis and chronic disease and augmented blood flow in acute disease. The apparent conflict between observations of prominent blood vessels,[68–70] increased blood flow,[71,72] and colonic ischemia[73] in studies of human and experimental colitis was addressed recently in a study of structural adaptations of murine colonic microvascular bed in the model of acute 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis.[74] The induction of TNBS colitis in this study was associated with a significant increase in the diameter of the mucosal plexus. The investigators observed that the increase in microvessel diameter coincided with the peak of the perivascular mononuclear infiltrate, that the dilatation of the mucosal plexus was associated with a significant reduction in flow velocity during mucosal transit, and that these structural changes showed a spatial association with the mononuclear infiltrate in the mucosal plexus. These data indicate that structural changes in colonic microcirculation are functionally associated with mononuclear cell transmigration and that vascular prominence, increased volumetric flow, and decreased flow velocity can coexist in the acute inflammatory response to TNBS.[74] Whether the same structural alterations are present in the chronic inflammation models remains to be determined.

The poorly healing, refractory inflammatory ulceration and damage in the IBD intestine strongly suggest that vasodilatory microvascular dysfunction results in tissue hypoperfusion. Hatoum and colleagues[75] examined the vasodilatory capacity of human intestinal microvessels by measuring *in vitro* vasodilatory response to acetylcholine (ACh) from pressurized submucosal intestinal arterioles rapidly isolated from resected gut specimens. Normal intestinal microvessels vasodilate in response to ACh using NO- and cyclooxygenase (COX)-dependent mechanisms, whereas chronically inflamed IBD arterioles demonstrate a diminished vasodilatory capacity. The decrease of vasodilatory capacity in chronically inflamed IBD microvessels is directly related to a loss of NO-dependent function, and these same vessels were found to be heavily dependent on COX to maintain their vascular tone (Figure 1, box 5). This microvascular endothelial dysfunction was associated with excess levels of oxidative stress and was specific for the vessels derived from IBD intestine, whereas it was not present in vessels isolated from the normal intestine, uninvolved areas of IBD bowel, and non-IBD acute inflammation. Another study demonstrates the diminished capacity of colonic arterioles to respond to endogenous endothelium-dependent vasodilators like bradykinin and shows that NAD(P)H oxidase-derived superoxide plays an important role in the inflammation-induced arteriolar dysfunction.[76] In addition, the fibrinoid occlusions appearing in CD-involved intestine have a histological appearance characteristic of prolonged disruption in the local vascular supply leading to microinfarction.[77] Taken together, these studies suggest that the microvascular anatomy undergoes vascular remodeling resulting in hypoperfused and ischemic/hypoxic environment in the gut, which possibly results in tissue necrosis. It is possible to argue that alterations of vascular anatomy and vascular dysfunction play a role in the pathogenesis of chronic inflammatory lesions, with the extent of vascular damage correlating with the severity of intestinal injury. Moreover, intrinsic alterations in the chronically inflamed and remodeled microcirculation underlie vascular dysfunction that seems to play a fundamental role in chronic, deregulated inflammation, which characterizes IBD.

Angiogenesis in IBD

One of the most novel aspects that directly implicate endothelial participation in inflammation is the process of angiogenesis.[78,79] It is now well established that angiogenesis and microvascular remodeling are intrinsic components of the tissue remodeling in chronic inflammatory diseases. Both processes result from EC proliferation and

often occur together, although they represent distinct phenomena in response to different stimuli. Angiogenesis is the growth of new capillary blood vessels from existing ones, whereas microvascular remodeling involves structural alterations such as enlargement of arterioles, capillaries, or venules without the formation of new vessels.[80] Inflammation and angiogenesis are intertwined in a number of ways. Inflammatory tissue is often hypoxic, and hypoxia is an important proangiogenic stimulus, acting through up-regulation of factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), TNF-α, hypoxia-inducible factor-1 (HIF-1), and other factors (Figure 1, box 6).[81,82] Extravasated plasma fibrinogen is involved in stimulation of neovascularization.[83] Inflammatory cells such as macrophages, lymphocytes, mast cells, and fibroblasts produce diverse angiogenic factors that stimulate vessel growth.[84,85] Finally, shear stress on the endothelium due to increased blood flow may stimulate angiogenesis.[86,87] Initially, functional changes prevail, including dilation, increased permeability, activation of the endothelium, and diapedesis. In the second phase, structural changes occur, with capillary and venule remodeling and proliferation of ECs.[88] In chronic inflammatory disorders, tissue damage and repair continue concurrently.[89] With time, the ECs in the inflamed capillaries respond to locally produced angiogenic factors and start to multiply, with these newly formed remodeled vessels ultimately becoming permanent.[89] The anatomical expansion of the microvascular bed combined with its increased activation state can now foster further influx of more inflammatory cells, and angiogenesis and inflammation become chronically codependent processes.

When ECs are involved in angiogenesis, they display a cell surface molecular pattern not found on resting vessels. Studies on intestinal biopsies from IBD patients demonstrated alterations of endothelial adhesion molecules,[90,91] as well as elevated levels of soluble adhesion molecules,[92] indicating endothelial activation. The hallmark of an angiogenic vessel is the expression of certain integrins, particularly $\alpha v\beta 3$ and $\alpha v\beta 5$, as well as up-regulation of several receptors for angiogenic factors.[78,88,93] Initial evidence for the involvement of angiogenesis in IBD was obtained from animal models of colitis. Intense mucosal neoangiogenesis that increased in parallel with disease progression was found in IL-$10^{-/-}$ colitic mice.[94] Blockade of the murine angiogenic endothelial marker $\alpha v\beta 3$ by ATN161, an antiangiogenic 5-mer peptide that binds to $\alpha 5\beta 1$ and $\alpha v\beta 3$, effectively decreased both neoangiogenesis and inflammation in the $CD4^+CD45RB^{high}$ colitis model of IBD.[95] In DSS-induced colitis in rats after the withdrawal of DSS, the disease activity gradually subsided, and hepatocyte growth factor (HGF) expression was significantly enhanced along with the augmented expression of IL-1β, TNF-α, and COX-2, accompanied by an increased number of proliferating ECs in the colon.[96]

Neoangiogenesis in CD and UC has been recently investigated by quantifying mucosal vascularization state, assessing local expression of the neoangiogenic marker $\alpha v\beta 3$, and exploring the presence of functional proangiogenic activity of IBD tissue.[97] Expression of $\alpha V\beta 3$ by the mucosal microvasculature resulted most prominently in mucosa affected by active inflammation. Moreover, $\alpha v\beta 3$ up-regulation in HIMEC cultures exposed to TNF-α, VEGF, and bFGF, all of which are increased in IBD tissue, supports the assumption that this phenomenon is due to the exposure of the endothelium to the proinflammatory and proangiogenic milieu of the neighboring tissue and further suggests that proinflammatory and proangiogenic factors act in a complementary fashion.[97] In addition, mucosal extracts from both CD and UC exhibited augmented capacity to induce a dose-dependent migration of HIMECs, indicating that locally produced angiogenic factors are biologically active. In addition, promoting angiogenesis in experimental IBD appears to worsen the disease, based on preliminary results with VEGF gene transfer in DSS colitis, which show a dramatic worsening of inflammation (S.D., unpublished observations). Along these lines is also the observation that CD146, a novel marker of endothelial junction remodeling, is highly expressed on ECs in intestinal biopsies from both CD and UC, with a decrease in the soluble form of CD146 in relation to active disease.[98] In addition, increased serum and/or tissue levels of several proangiogenic factors have been reported in patients with active IBD.[99–102] The notion that immune-nonimmune interactions are important for the maintenance and propagation of inflammation-induced mucosal angiogenesis is substantiated by recent studies that identify the critical role of the CD40-CD40L pathway in immune-driven angiogenesis.[94,103] Indeed, inflammation-activated CD40L-expressing T cells might trigger intestinal fibroblast activation and angiogenic cytokine release, in turn causing activation of HIMEC angiogenesis. In addition, soluble CD40L directly fosters mucosal angiogenesis, pointing to a dual mechanism responsible for CD40-dependent angiogenesis in the inflamed gut.[94]

The relationship between inflammatory and angiogenic responses in experimental colitis governs the regulation of mediators that exert control over the angiogenic process, enabling a vicious cycle of disease activity. A recent report showed that high concentrations of angiogenic cytokines, such as VEGF-A, increase leukocyte interactions with colon microvascular ECs in a manner similar to that of proinflammatory agents such as TNF-α.[104] Using DSS-induced and $CD4^+CD45RB^{high}$ T-cell transfer models of colitis, Chidlow and colleagues[95] demonstrated that increased angiogenic activity in response to chronic inflammation plays an important pathophysiological role during experimental colitis. In both colitis models, an up-regulation of certain proangiogenic mediators, such as matrix metalloproteinase-2 and -9 (MMP-2, MMP-9), endothelial sphingolipid G-protein-coupled receptor 1 (Edg1), endoglin, prostaglandin-endoperoxide synthase 2 (COX2), TNF-α, chemokine (CXC) ligand 1 (Gro1), and HGF, and down-regulation of a few anti-angiogenic factors, including CD36 antigen and chromogranin A, were observed. Interestingly, the authors found differential regulation of numerous angiogenic genes and anti-angiogenic or angiostatic genes between the two models, suggesting that angiogenesis may primarily occur

through loss of angiogenic inhibition in the DSS model, whereas angiogenesis in the $CD4^{+}CD45RB^{high}$ model likely occurs because of dramatic differential up-regulation of proangiogenic mediators. These data indicate interventions aimed at increasing anti-angiogenic activity versus blockade of proangiogenic mediators as possible selective means for treating various forms of IBD.

In summary, the available data denote enhancement of angiogenesis in IBD and highlight angiogenesis as a major contributor to the initiation and perpetuation of chronic intestinal inflammation.

Concluding Remarks

The complex nature of IBD and our still limited knowledge of its cause(s) and perpetuation mechanisms render treatment a great clinical challenge. The heterogeneity of patient responses to treatment likely reflects the heterogeneity of underlying inflammatory mechanisms, suggesting that multiple therapeutic approaches should be taken into consideration and pointing toward combined treatment as a possible solution. The major contribution of microvasculature to the pathogenesis of intestinal inflammation, uncovered over the past few decades, implies that intestinal endothelium is a possible critical target in treatment of IBD. Biological agents targeting adhesion molecules and chemokines to reduce leukocyte infiltration and inflammatory responses, modulation of coagulation pathways, angiogenesis inhibition, and reversal of microvascular dysfunction are all currently areas of intense study with the goal to rapidly expand our therapeutic armamentarium for IBD therapy and to translate bench research and conquests to the patient bedside.[105]

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