The vascular contribution in the pathogenesis of inflammatory bowel disease

Ossama A. Hatoum, Hiroto Miura, and David G. Binion

Division of Cardiovascular Medicine and Division of Gastroenterology and Hepatology, Department of Medicine, Cardiovascular Research Center, and Digestive Disease Center, Froedtert Memorial Lutheran Hospital, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

The two major forms of human 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 the majority of research into IBD pathogenesis has focused on immune dysregulation, vascular involvement in IBD has also been recognized over the past four decades, as these disorders have been associated with hypercoagulability and vasculitis. Advances in vascular biology have delineated a central role for the microcirculation in the initiation and perpetuation of the inflammatory process. Investigation in the molecular and cellular mechanisms underlying human IBD has demonstrated an important role for the intestinal microvascular endothelium in both mucosal immunity as well as the chronic inflammation that characterizes IBD. Chronically inflamed microvessels and endothelial cells in the setting of IBD show significant alterations in physiology and function compared with microvessels and cells from uninvolved the intestine, where IBD microvessels demonstrate an enhanced capacity to adhere leukocytes. Understanding of leukocyte-endothelial interaction in IBD is presently leading to new antiadhesion molecule agents that target the vasculature for therapy.

The intestinal microvasculature may contribute to impaired mucosal healing in CD and UC as both forms of IBD are characterized by refractory mucosal ulceration and damage. Previous work has demonstrated an abnormal, remodeled intestinal vascular architecture characterized by stenotic microvessels and significantly decreased mucosal perfusion, which may underlie chronic ischemia and impaired wound healing in IBD. We review the role of the vasculature in the pathogenesis of human IBD, focusing on hypercoagulability, tissue ischemia, and a proinflammatory phenotype in the intestinal microvascular endothelium.

The systemic vasculature in IBD pathogenesis

Hypercoagulability and thrombosis in IBD

IBD is recognized to be a hypercoagulable state, and thromboembolic disease is a significant cause of morbidity and mortality in patients with both CD and UC. Deep venous thrombosis and pulmonary embolism are the most frequent thromboembolic manifestations, which readily explains the high rates of morbidity and mortality associated with these complications (70, 73). Hypercoagulability in IBD has been attributed to a variety of factors including thromboembosis as well as increased levels of clotting factors V and VIII, fibrinogen (52–54), accelerated thromboplastin generation, acquired antithrombin III deficiency (15, 37, 72), and decreased protein C and protein S (1, 2, 44, 47). However, no single consistent coagulation abnormality has been identified (45, 57). Other potential mechanisms include the presence of anti-phospholipid antibodies and lupus anticoagulant invoking the involvement of autoimmune mechanisms (16, 18, 80). Elevated anti-cardiolipin antibodies have been detected in some IBD patient populations (3, 48, 49). A more recent study (79) has focused on elevation in plasma homocysteine in IBD patients, which may also contribute to underlying hypercoagulability.

It is not known whether hypercoagulability in IBD is a secondary phenomenon associated with chronic intestinal inflammation or may represent an underlying, mechanistic factor that contributes to disease pathogenesis. The potential protective effect of an underlying bleeding diathesis in preventing the development of IBD was investigated by Thompson et al. (75), 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 diathesis exerted a protective effect against development of IBD, suggesting an important role of inappropriate thrombosis and vascular occlusion in the pathogenesis of human IBD.

The potential pathogenic contribution of thrombosis in IBD pathogenesis has been explored through the use of anticoagulation therapy in the treatment of IBD patients. Heparin treatment of thromboembolic complications in IBD patients resulted in clinical improvement in their bowel disease in open-label as well as randomized clinical trials (28, 47, 52) using both unfractioned (25, 27) and low-molecular-weight heparin (76). We previously reported the therapeutic success of unfractioned heparin therapy in a female CD patient who experienced a refractory colitis flare during the first trimester of pregnancy, an additional major risk for hypercoagulability. Whether this treatment modality exerted direct antithrombotic effects or potentially...
exerted alternative anti-inflammatory effects is not known (67).

Vasculitis, Atherosclerosis, and IBD Pathogenesis

The association between IBD and systemic vasculitis is well known and includes necrotizing vasculitis of the skin (7), lung, and penis; chronic polyneuropathy; iritis; polyserositis; cerebral vasculitis (62); Takayasu’s arteritis (71); and retinal vasculitis, a particularly devastating ocular complication (21). Examination of the resected intestine of CD patients has found evidence of granulomatous microvasculitis (82), which suggests common pathophysiological mechanisms (29, 46).

The worldwide distribution of IBD has paralleled the incidence and prevalence of atherosclerosis, targeting primarily Western populations over the second half of the 20th century. Recent investigation into mechanisms underlying atherosclerosis has focused on the potential contribution of inflammation in vascular disease (61). Data from population-based studies suggest that patients suffering from either CD or UC will experience increased rates of atherosclerosis and ischemic heart disease at earlier ages in both males and females compared with age- and sex-matched controls (64, 65).

The correlation between atherosclerosis and IBD has prompted investigation of potential shared molecular and cellular mechanisms contributing to pathogenesis. The role of dietary lipids and lipoproteins in vascular disease is a well-established mechanism that is believed to contribute to atherosclerosis. We (10) have previously demonstrated that oxidized lipoproteins induce activation of microvascular endothelial cells isolated from the human intestine, leading to increased expression of cell adhesion molecules (CAMs) and chemokines that mediate leukocyte adhesion, a critical early step in both inflammation and atherosclerosis. An additional potential mechanism that may be shared in the pathogenesis of both atherosclerosis and IBD involves CD40 and its ligand (CD40L), a molecule that is felt to mediate thrombosis, inflammation, and vascular remodeling. Urbich et al. (77) suggested that the CD40 pathway is involved in the vascular restenotic process, as CD40L is found in thrombi developing on the surface of atherosclerotic plaques (4, 38). Danese et al. (19) demonstrated that IBD patients express CD40L in their circulating platelets and the inflamed mucosa, where microthrombosis may be a prominent feature, further propagating an inflammatory response and may also contribute to vessel remodeling, which is a common feature in both atherosclerosis and IBD (77, 81).

THE INTESTINAL CIRCULATION AND MICROVASCULATURE IN IBD: CONTRIBUTION TO GUT ISCHEMIA

The Splanchnic Circulation and Intestinal Angiography

The human bowel is highly vascularized, receiving a significant proportion of cardiac output, which varies in response to physiological need. At rest, intestinal perfusion via the superior mesenteric artery will range from 29 to 70 ml·min⁻¹·100 g intestinal tissue⁻¹ (33, 41–43), whereas in the fed state, splanchnic hyperemia increases perfusion by 28–132% (33). Angiographic studies of the IBD intestine performed over the past three decades have demonstrated preserved anatomy in the superior and inferior mesenteric arteries, with significant disease-related abnormalities in the vasa recta, which correspond with disease extent and severity. In early IBD, angiographic studies (35, 40, 59, 60) have demonstrated tortuous, dilated vessels, bizarre distribution, and small luminal irregularities in the peripheral branches, together with loss of normal tapering, right-angle bifurcations, and terminal coiling as the vessels penetrate the bowel wall. In contrast, advanced IBD lesions demonstrate reduced vessel diameter (24, 55, 56), decreased vascular density, and diminished blood flow in the involved segments (12). These angiographic studies suggest that a relative lack of perfusion emerges in the course of chronic inflammation in IBD.

Intestinal Microvascular Perfusion

Measurements of intestinal blood flow using direct and indirect methods have characterized a loss of intestinal perfusion, which emerges during the progression of chronic inflammation in IBD. With the use of intraoperative isotope washout techniques (43), in vivo abdominal angiography, and endoscopic Doppler flowmetry (5, 54), distinct patterns of vascular perfusion have been correlated with discrete phases of both CD and UC. Early fulminant 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 (6). These observations have been confirmed in a subsequent study (5), where the most severe decrease in vascular perfusion was found in association with fibrotic strictures. This potential contribution of ischemia to IBD chronic inflammation was evaluated by Wakefield et al. (82) using scanning electron micrographs of corrosion microcasts after bowel resection; they identified occlusive fibrinoid lesions in the arteries supplying areas of the intestine affected by CD, which were not found in uninvolved areas of bowel. The identification of microvascular damage was demonstrated as an early pathological finding that preceded the development of mucosal ulceration. The interrelationship among vascular perfusion, tissue homeostasis, and wound healing has prompted evaluation of intestinal perfusion in the setting of human IBD. The poorly healing, refractory inflammatory ulceration and damage in the IBD intestine strongly suggest that microvascular dysfunction with diminished vasodilatory capacity will result in tissue hypoperfusion. Hatoum et al. (36) examined vasodilator responses in human intestinal microvessels by measuring in vitro vasodilatory response to ACh from pressurized submucosal intestinal arterioles (50–150 μm in diameter) rapidly
isolated from resected gut specimens. Normal intestinal microvessels vasodilate in response to ACh using nitric oxide (NO)- and cyclooxygenase (COX)-dependent mechanisms, whereas chronically inflamed IBD arterioles (both CD and UC) demonstrated a diminished vasodilatory capacity (maximum dilation: 81/100 vs. 16/100 in IBD arterioles, \( P < 0.05 \)). This decreased vasodilatory capacity in chronically inflamed IBD microvessels was 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. This microvascular endothelial dysfunction was associated with excess levels of oxidative stress, which was not present in vessels isolated from the normal intestine, uninvolved areas of IBD bowel, and non-IBD acute inflammation (Fig. 1) (36).

MICROVASCULAR ENDOTHELIUM IN IBD PATHOGENESIS: CONTRIBUTION TO LEUKOCYTE RECRUITMENT IN INFLAMMATION

Human Intestinal Microvascular Endothelial Cells in IBD Pathogenesis

Endothelial cells play an early and rate-limiting step in the inflammatory process. Pioneering work by Bevilacqua et al. (9) demonstrated that endothelial activation in response to cytokines and inflammatory mediators was a critical step in circulating leukocyte recruitment. Initial investigation into the potential role of endothelial cells in IBD pathogenesis focused on histological evaluation, characterizing the morphology of the microvasculature in chronically inflamed bowel. Using transmission electron microscopy, Dvorak et al. (23) demonstrated abnormalities in endothelial cells from the CD gut microcirculation that included loss of endothelial monolayer integrity with tissue edema, extravasation of red blood cells, focal venular endothelial necrosis, and endothelial cell hypertrophy.

Subsequent work has focused on intestinal microvascular endothelial cells and their expression of CAMs, which play a major role in mucosal leukocyte recruitment (31, 66). Immunolocalization of CAMs demonstrated a marked increase in E-selectin and intercellular adhesion molecule (ICAM)-1 expression in the IBD intestine, whereas vascular CAM (VCAM)-1 expression was less clearly demonstrated. Investigation by Briskin et al. (13) has demonstrated an increase in the gut-specific homing molecule mucosal addressin CAM-1, which plays a major role in the recruitment of leukocytes expressing \( \alpha_4 \)-integrin into the mucosal immune compartment (13).

Studies investigating potential alterations in leukocyte homing patterns in IBD were carried out by Salmi et al. (69). This work demonstrated that naïve lymphocytes were recruited by the IBD intestinal microvascular endothelium compared with control gut microvessels, which preferentially bound memory lymphocytes. These findings were confirmed by Burgio et al. (14), who also demonstrated an altered pattern of leukocyte binding in CD, where naïve monocytes and T cells were again preferentially recruited to the chronically inflamed intestine.

To more fully define the contribution of microvascular endothelial cells in chronic intestinal inflammation, our laboratory (10, 31, 32, 51) developed protocols for the routine isolation and long-term culture of human intestinal microvascular endothelial cells (HIMECs). HIMECs isolated from both chronically inflamed CD and UC intestines demonstrated a significantly enhanced capacity to adhere...
leukocytes compared with control HIMECs, a phenomenon that was only elicited after activation with cytokines (IL-1β and TNF-α) and bacterial LPS and was not present in unstimulated cells. Leukocyte “hyperadhesion” was only present in chronically inflamed IBD HIMECs, as cultures derived from uninvolved areas in close proximity failed to demonstrate increased leukocyte binding (11).

The mechanisms underlying leukocyte hyperadhesion in the chronically inflamed IBD HIMECs failed to show alterations in patterns of CAM expression between normal and IBD HIMECs. Investigation shifted to focus on NO generation in HIMECs, an alternate pathway that would influence the activation status of these tissue-specific endothelial cells, and their capacity to bind circulating leukocytes (50). 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 HIMECs demonstrated a loss of iNOS gene expression after activation that corresponded with diminished NO generation and enhanced leukocyte binding.

**Anti-adhesion Molecule Therapy in IBD**

Targeting endothelial-leukocyte interaction for therapeutic benefit in patients with IBD has received intense interest as three experimental agents have been evaluated in controlled trials (78). ICAM-1 expression in CD patients was targeted using alicaforsen (ISIS 2302, ISIS Pharmaceuticals; Carlsbad, CA) (22, 68, 20, 58), a phosphorothioate oligodeoxynucleotide designed to specifically hybridize to the 3'-untranslated region of human ICAM-1 mRNA, which leads to rapid degradation and the prevention of translation (8, 17, 39, 63, 63). Initial clinical attempts using alicaforsen in CD treatment demonstrated a steroid-sparing effect and induced remission in up to 40% of patients (83). Subsequent clinical trials have not repeated this level of success, but studies with increased dosages of this agent continue at present.

Early investigation of leukocyte-endothelial interaction in IBD focused on leukocyte α4β1-integrin, which plays a major role in mucosal immune homing to the intestinal microvascular endothelium and has demonstrated therapeutic potential in ameliorating the chronic colitis that develops spontaneously in the cotton-top tamarin (66). The success of anti-α4β1-integrin treatment with monoclonal antibodies in this primate model of IBD has provided the rationale for developing human trials of this strategy. Monoclonal antibodies targeting leukocyte expression of α4β1-integrin include the humanized anti-α4β1-integrin antibody natalizumab (Antegren, Elan Pharmaceuticals; San Diego, CA) as well as LDP-02 (Millenium Pharmaceuticalsl Cambridge, MA), both of which continue in clinical trials at the present time (26, 30).

**SUMMARY AND FUTURE DIRECTIONS**

The vasculature plays a central role in human IBD and may contribute to pathogenesis through thrombotic, ischemic, or inflammatory mechanisms. Investigation has defined an altered microvascular anatomy in the affected IBD bowel that corresponds with diminished perfusion in the setting of chronic, longstanding inflammation. The intestinal microvasculature exposed to repeated injury and repair undergoes extensive remodeling that corresponds with impaired vasoperfusion as well as enhanced and sustained inflammatory activation. This is linked to impaired wound healing and may underlie the refractory, mucosal ulceration that is the hallmark of both CD and UC (Fig. 2).

Investigation of microvascular dysfunction in IBD will provide important insights into the microvascular biology and physiology of chronic inflammatory disease. Our understanding of the role of the vasculature in IBD pathogenesis is also paving the way for the development of novel strategies targeting the microvasculature, specifically endothelial-leukocyte interaction with the development of antiadhesion molecule biological agents for clinical use.

**REFERENCES**


56. Lunderquist A and Lunderquist A.

58. Markowitz RL, Ment LR, and Gryboski JD.

60. Mellor JA, Chandler GN, Chapman AH, and Irving HC.

61. Murray DR and Freeman GL.


