VEGF-A Links Angiogenesis and Inflammation in Inflammatory Bowel Disease Pathogenesis

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Background & Aims: **Vascular endothelial growth factor A (VEGF-A) mediates angiogenesis and might also have a role in inflammation and immunity. We examined whether VEGF-A signaling has a role in inflammatory bowel disease (IBD).** ***Methods:*** **Expression levels of VEGF-A, and its receptors VEGFR-1 and VEGFR-2, were examined in samples from patients with IBD and compared with those of controls. The capacity of VEGF-A to induce angiogenesis was tested in human intestinal microvascular endothelial cells using cell-migration and matrigel tubule-formation assays. Levels of vascular cellular adhesion molecule-1 and intercellular adhesion molecule were measured by flow cytometry to determine induction of inflammation; neutrophil adhesion was also assayed. Expression patterns were determined in tissues from mice with dextran sulfate sodium (DSS)-induced colitis; the effects of VEGF-A overexpression and blockade were assessed in these mice by adenoviral transfer of VEGF-A and soluble VEGFR-1. Intestinal angiogenesis was measured by quantitative CD31 staining and leukocyte adhesion in vivo by intravital microscopy.** ***Results:*** **Levels of VEGF-A and VEGFR-2 increased in samples from patients with IBD and colitic mice. VEGF-A induced angiogenesis of human intestinal microvascular endothelial cells in vitro as well as an inflammatory phenotype and adherence of neutrophils to intestinal endothelium. Overexpression of VEGF-A in mice with DSS-induced colitis worsened their condition, whereas overexpression of soluble VEGFR-1 had the opposite effect. Furthermore, overexpression of VEGF-A increased mucosal angiogenesis and stimulated leukocyte adhesion in vivo.** ***Conclusions:*** **VEGF-A appears to be a novel mediator of IBD by promoting intestinal angiogenesis and inflammation. Agents that block VEGF-A signaling might reduce intestinal inflammation in patients with IBD.**

Inflammatory bowel disease (IBD) pathogenesis involves the interplay of multiple biologic components, among which nonimmune cells play a crucial role.[1–3] In particular, endothelial cells play a key role in multiple aspects of chronic intestinal inflammation, including expression of cell adhesion molecules (CAM) and chemokine secretion, recruitment of leukocytes and platelets, acquisition of a prothrombotic phenotype, and through immune-driven angiogenesis.[4,5] Angiogenesis is therefore a complex process mediated by multiple cell types and mediators[6,7] and is fundamental to many biologic processes, including growth, development, and repair.

Besides its well-known role in cancer, it has become clear that angiogenesis is also an integral component of a diverse range of nonneoplastic chronic inflammatory and autoimmune diseases, including atherosclerosis, rheumatoid arthritis, diabetic retinopathy, psoriasis, airway inflammation, peptic ulcers, and Alzheimer's disease.[6,8,9] Indeed, angiogenesis is intrinsic to chronic inflammation and is associated with structural changes, including activation and proliferation of endothelial cells, and capillary and venule remodeling, all of which result in expansion of the tissue microvascular bed.[10–12] A potential functional consequence of this expansion is the promotion of inflammation through various correlated mechanisms. First, influx of inflammatory cells may increase; second, there is an increased nutrient supply to the metabolically active immune process; and, third, the activated endothelium contributes to the local production of cytokines, chemokines, and matrix metallopro-

Abbreviations used in this paper: CD, Crohn's disease; CAM, cell adhesion molecules; HIMEC, human intestinal microvascular endothelial cell; ICAM, intercellular adhesion molecule; UC, ulcerative colitis; VCAM, vascular cellular adhesion molecule; VEGF-A, vascular endothelial growth factor A; VEGFR, VEGF receptor.

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teinases.[13,14] The anatomic expansion of the microvascular bed combined with its increased functional activation can therefore foster further recruitment of inflammatory cells, and angiogenesis and inflammation become chronically codependent processes.[10,12,14,15] In addition, many of the mediators that are fundamental players in angiogenesis are also inflammatory molecules.[16,17]

The angiogenic role played by the pathways involving the vascular endothelial growth factors (VEGFs) and their receptors is well characterized. There are 7 members of the VEGF family, ie, VEGF-A, -B, -C, -D, -E, -F, and placental growth factor, and these each interact with specific receptors, such as VEGFR-1 (flt-1), VEGFR-2 (KDR), and VEGFR-3.[18,19] VEGF-A is the best characterized[7,20,21] and is a fundamental mediator of pathologic angiogenesis, such as in neoplasia and chronic inflammation. Indeed, targeted blockade of VEGF-A is currently being used as a therapeutic approach to block angiogenesis in malignant tumors.[22,23]

VEGF-A is crucially involved in several chronic inflammatory disorders,[24–28] in which VEGF-A not only promotes pathologic angiogenesis but directly fosters inflammation.[7,18,25,26] It is now well established that, in diseases such as rheumatoid arthritis, psoriasis, atherosclerosis, and chronic lung inflammation, VEGF-A is intimately involved in disease pathogenesis, and targeting VEGF-A is a promising new therapeutic strategy to dampen inflammation.[7,9,18,27–32]

Studies from our laboratory and others have shown that angiogenesis is a novel component of both ulcerative colitis (UC) and Crohn's disease (CD) and that targeting angiogenesis by integrin $\alpha v\beta 3$ blockade is an effective and entirely novel approach to block experimental colitis.[33–36] However, the specific mediators involved in immune-driven angiogenesis associated with IBD are still poorly defined.[37]

A few reports have described overexpression of VEGF-A in humans with IBD,[4,37] but the functional significance of such up-regulation is not yet understood. In addition, the messenger RNA (mRNA) for VEGF-A is strongly up-regulated in animals with chronic experimental colitis.[35] In murine colonic-derived endothelial cells, VEGF-A triggers an inflammatory phenotype by up-regulating CAMs and inducing adhesion of neutrophils and T cells, thus supporting an inflammatory role for this cytokine in the intestine.[38] However, thus far, VEGF-A and its receptors have not been fully characterized in patients with IBD nor has the functional role of VEGF-A been studied in these patients.

We have therefore evaluated the role of the VEGF-A pathway[39] in the pathogenesis of IBD. Here, we show that VEGF-A is up-regulated in involved tissues in humans with IBD and colitic mice, as is its receptor VEGFR-2, but not VEGFR-1. In vitro, VEGF-A induces both angiogenic activity and an inflammatory phenotype in human intestinal microvascular endothelial cells (HIMEC), whereas overexpression in vivo increases disease severity and blockade decreases disease severity in colitic mice. This in vivo effect correlated with increased or decreased angiogenesis, respectively. In addition, VEGF-A induced recruitment of leukocytes to the inflamed intestine in vivo, thus fostering inflammation. These results strongly support the important role played by the VEGF pathway in both inflammation and the angiogenesis that underlies disease pathogenesis in IBD.

Materials and Methods

For additional information on materials and methods, see supplementary materials and methods section (see supplementary materials and methods online at www.gastrojournal.org).

Patient Population

Patients with active and inactive CD and UC were studied, and healthy individuals were enrolled as controls. Patients and controls were recruited at the Division of Gastroenterology, Istituto Clinico Humanitas, Milan, Italy, and the study was approved by the Institutional Review Board. Ethical guidelines were followed by the investigator in studies on humans or animals and described in the paper. Clinical disease activity was assessed by the Harvey–Bradshaw Activity Index and the Colitis Activity Index, as previously reported.[33] All diagnoses were confirmed by clinical, radiologic, endoscopic, and histologic criteria.

Immunostaining of Mucosal Expression of VEGFR-1 and -2 in Human and Murine Colonic Tissues and CD31 in Murine Colonic Tissues

Immunostaining was performed as previously described[40] (see supplementary materials and methods online at www.gastrojournal.org).

Isolation and Culture of HIMEC

HIMEC were isolated as previously described[41] (see supplementary materials and methods online at www.gastrojournal.org).

Western Blotting Analysis

Immunoblotting was performed as previously described[42] (see supplementary materials and methods online at www.gastrojournal.org).

Tubule Formation and Migration Assay

Endothelial cell tube formation was assessed using Matrigel (BD Biosciences, San Jose, CA), as previously described[36] (see supplementary materials and methods online at www.gastrojournal.org). Chemotaxis was assessed as previously reported[40,43] (see supplementary materials and methods online at www.gastrojournal.org).