Negative Regulators of Angiogenesis in Inflammatory Bowel Disease: Thrombospondin in the Spotlight

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Key Words

Crohn's disease · Endothelium · Inflammatory bowel disease · Ulcerative colitis

Abstract

Angiogenesis is the growth of new blood vessels. In the two major forms of inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis, robust angiogenesis exists, and its blockade may have therapeutic potential, as shown in animal models of experimental intestinal inflammation. While abundant literature is available on the positive regulators of intestinal pathological angiogenesis, e.g. VEGF, b-FGF, IL-8, CD40 and CD40L, almost no data exist on negative regulators. Thrombospondin-1 is a negative regulator of angiogenesis, and it plays a new role in IBD-associated angiogenesis. In addition, recombinant thrombospondin-1 may inhibit pathological angiogenesis and may offer a new therapeutic approach to intestinal inflammation.

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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, our understanding of the pathogenic mecha-

nisms underlying intestinal inflammation has been expanded [1]. In particular, the importance of vascular involvement in IBD has been fully recognized recently [2]. It is now clear that the abnormalities underlying the pathogenesis of IBD are not restricted to those mediated by classical immune cells, such as T and B lymphocytes, macrophages and dendritic cells, but also involve nonimmune cells. Advances in vascular biology have delineated a central role for the microcirculation in the initiation and perpetuation of the inflammatory process [3].

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 the flow of nutrients and blood, tissue hemostasis, and cell trafficking and distribution, as well as pathological processes such as inflammation [3]. Endothelial cells (EC) play a key role in intestinal 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 the pathogenesis of inflammation [4]. Indeed, the vascular response is a key component of inflammation, where tissue EC become activated displaying a functional phenotype, which includes leakiness, leukocyte adhesiveness, procoagulant activity and eventually angiogenesis [3]. Endothelial cells are able to amplify and maintain the inflammatory response by angiogenesis.

Novel aspects on the endothelial participation in the inflammatory process triggering angiogenesis have been reported recently [5]. It is now well established that angiogenesis and microvascular remodeling are intrinsic components of tissue remodeling in chronic inflammatory diseases [6]. 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, usually enlargement of arterioles, capillaries or venules without the formation of new vessels [6]. 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), tumor necrosis factor (TNF)- α , hypoxia-inducible factor and other factors. Inflammatory cells, such as macrophages, lymphocytes, mast cells and fibroblasts produce diverse angiogenic factors that stimulate vessel growth [5, 7–9]. 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 EC. In chronic inflammatory disorders, tissue damage and repair continue concurrently. With time, the EC in the inflamed capillaries respond to locally produced angiogenic factors and start to multiply, and ultimately these newly formed remodeled vessels become permanent. 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 co-dependent processes [5, 7].

When EC 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 in endothelial adhesion molecules as well as elevated levels of soluble adhesion molecules, indicating endothelial activation [10, 11]. 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 [12]. 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 [13]. Blockade of the murine angiogenic endothelial marker $\alpha v \beta 3$

by ATN161 effectively decreased both neoangiogenesis and inflammation in this IBD model. In a rat model of dextran sodium sulfate (DSS)-induced colitis after DSS withdrawal, disease activity gradually subsided and hepatocyte growth factor expression was significantly enhanced concomitant with the augmented expression of IL-1 β , TNF- α and COX-2, and an increased number of proliferating EC in the colon [13].

Recently, neoangiogenesis in CD and UC has been investigated by quantifying mucosal vascularization, assessing local expression of the neoangiogenic marker $\alpha v \beta 3$ and exploring the presence of functional proangiogenic activity in IBD tissue [14]. In the microvasculature, expression of $\alpha v \beta 3$ was most prominent in mucosa affected by active inflammation. Moreover, $\alpha v \beta 3$ up-regulation in human intestinal microvascular EC (HIMEC) cultures exposed to TNF- α , VEGF and bFGF, all of which are increased in IBD tissue, support 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. In addition, mucosal extracts from both CD and UC exhibited augmented capacity to induce a dose-dependent migration in HIMEC, indicating that locally produced angiogenic factors are biologically active [14]. The notion that immune-nonimmune interactions are important for the maintenance and propagation of inflammation-induced mucosal angiogenesis has recently been substantiated by studies identifying a critical role of the CD40-CD40L pathway in immune-driven angiogenesis. Indeed, inflammation-activated CD40L-expressing T cells might trigger intestinal fibroblast activation and angiogenic cytokine release, causing, in turn, activation of HIMEC angiogenesis [15]. In addition, soluble CD40L directly fosters mucosal angiogenesis, pointing to a dual mechanism responsible for CD40-dependent angiogenesis in the inflamed gut.

The relationship between inflammatory and angiogenic responses in experimental colitis governs the regulation of mediators that control the angiogenic process, enabling a vicious cycle of disease activity. A recent report showed that high concentrations of angiogenic cytokines, e.g. VEGF-A, increase leukocyte interactions with colon microvascular EC similarly to proinflammatory agents such as TNF- α . Using DSS-induced and CD4+CD45RB^{high} T cell transfer models of colitis, Chidlow et al. [16] demonstrated that increased angiogenic activity in response to chronic inflammation plays an important pathophysiological role during experimental

colitis. 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 due to dramatic differential up-regulation of proangiogenic mediators.

In this issue of *Pathobiology*, Puneekar et al. [17] report on the functional role of thrombospondin-1 (TSP-1), a totally new molecule implicated in colitis-associated angiogenesis. Using two different approaches, TSP-1, a natural inhibitor of angiogenesis, was shown to play a role in angiogenesis in experimental IBD. (1) TSP-1 knockout mice displayed an increased susceptibility to DSS-induced colitis and had a more robust inflammatory-driven angiogenic response, and (2) treatment with an anti-angiogenic TSP-mimetic peptide resulted in amelioration of experimental IBD.

The data presented in their study are novel and important, since they deal with a new topic in intestinal angiogenesis. Their study helps to elucidate the functional role of natural angiogenesis inhibitors in IBD. In particular, new light is shed on the importance of investigating negative regulators of angiogenesis in IBD, in addition to the

classical approach studying the positive regulators of neovascularization. Although only murine data are presented, it is highlighted that TSP-1 is functionally relevant in experimental intestinal inflammation. Indeed, TSP-1 knockout mice have a more pronounced susceptibility to experimental IBD and an increased inflammation-associated pathological angiogenesis. More importantly, their results pave the way for a new therapeutic approach to IBD management. Indeed, the authors show that by using a TSP-1 mimetic peptide, disease activity is improved, thus suggesting that TSP-1 may be a new target for therapeutic interventions, and that tipping the balance between positive and negative regulators of angiogenesis could be of importance in the management of IBD. Studies in humans should assess the functional role of TSP-1 in both forms of IBD, thus potentially leading to the development of novel angiogenesis inhibitors in human CD and UC.

Acknowledgments

This work was supported by a grant from the Broad Medical Research Program to S.D.

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