A novel pathogenic role for microvasculature in inflammatory bowel disease


Introduction

Crohn’s disease (CD) and ulcerative colitis (UC) are the two major forms of inflammatory bowel disease (IBD). Although their etiology is still unknown, the pathogenic mechanisms underlying intestinal inflammation have made impressive progress in our understanding. In particular, the abnormalities underlying IBD pathogenesis are not restricted to those mediated by classical immune cells such as T and B lymphocytes, macrophages and dendritic cells, but also nonimmune cells. Interestingly, endothelium has become one of the major areas of investigation in gut inflammation.

Abstract. – Crohn’s disease (CD) and ulcerative colitis (UC) are the two major forms of inflammatory bowel disease (IBD). Although their etiology is still unknown, the pathogenic mechanisms underlying intestinal inflammation have made impressive progress in our understanding. In particular, the abnormalities underlying IBD pathogenesis are not restricted to those mediated by classical immune cells such as T and B lymphocytes, macrophages and dendritic cells, but also nonimmune cells. Interestingly, endothelium has become one of the major areas of investigation in gut inflammation.

Key Words:
Crohn’s disease, Ulcerative Colitis, Inflammatory Bowel Disease, Angiogenesis.

Immune-Driven Pathological Angiogenesis

One of the very new aspects that directly implicate endothelial participation into inflammation is the process of angiogenesis. It is now very well established that angiogenesis and microvascular remodeling are elements of the tissue remodeling in chronic inflammatory diseases. Both types of change in the microvasculature result from endothelial cell proliferation and often occur together, but they represent different phenomena and responses to different stimuli. Angiogenesis is the growth of new blood vessels from existing ones, whereas microvascular remodeling involves structural alterations – usually enlargement – of arterioles, capillaries or venules, without the formation of new vessels. As inflammatory diseases evolve, the microvascu-
lature undergoes progressive changes in structure and function. Blood vessels enlarge or proliferate to supply nutrients to accumulations of inflammatory cells in chronically inflamed tissues. Changes in the microvasculature in chronic disease may be out of proportion to the increased metabolic needs of tissues because of the overproduction of growth factors that stimulate vessel growth and remodeling. The contribution of blood vessels to inflammation can be divided in two phases. In the first phase, functional changes prevail that include dilation, increased permeability, activation of the endothelium, and diapedesis. In the second phase, structural changes occur, with capillary and venule remodeling and proliferation of endothelial cells. In chronic and autoimmune disorders, infiltration by macrophages and lymphocytes ensues, and tissue damage and repair continue concurrently. With time, the endothelial cells in the inflamed capillaries multiply, and these newly formed and remodeled vessels are maintained. The anatomical expansion of the microvascular bed combined with its increased activation state fosters further influx of inflammatory cells, and angiogenesis and inflammation become chronically co-dependent processes.

Also, blood vessels in diseased tissues usually have multiple abnormalities, ranging from the expression of molecules not found on normal vessels to alterations in endothelial barrier function and leakiness. When endothelial cells are involved in angiogenesis, they display a cell surface molecular not found on resting vessels. The hallmark of an angiogenic vessel is the expression of certain integrins, particularly αvβ3 and αvβ5, characteristic of endothelial cell undergoing active proliferation. Furthermore, angiogenic vessels up regulate several receptors for various angiogenic factors. Angiogenesis in inflammation is a very complex biological process, and requires the coordinated involvement of multiple cells and growth factors. Up today, besides the classical major angiogenic factors such as VEGF, bFGF, TGF-β, and TNF-α, a large myriad of new angiogenic factors are daily reported, and these include growth factors, cytokines, matrix metalloproteinases, cell adhesion molecules, integrins and extracellular matrix components.

**Pathological Angiogenesis in IBD**

Neoangiogenesis, the growth of new blood vessels, is not only critical for cancer growth, but also several chronic inflammatory disorders, like rheumatoid arthritis, psoriasis and atherosclerosis. Whether neoangiogenesis also occurs in IBD has not been explored.

We investigated neoangiogenesis in Crohn’s disease (CD) and ulcerative colitis (UC) by quantifying mucosal vascularization state, assessing local expression of the neoangiogenic marker αvβ3, and exploring the presence of functional pro-angiogenic activity of IBD tissue. In vivo levels of the key angiogenic molecules IL-8, bFGF and VEGF, and their cellular source(s) were examined, and whether they could promote angiogenesis in vitro was tested with human intestinal microvascular endothelial cells (HIMEC). ELISA and immunoblotting measured IL-8, bFGF and VEGF in mucosal extracts, and in cultures of human intestinal fibroblasts (HIF) and lamina propria mononuclear cells (LPMC) stimulated by TNF-α, and LPS or anti-CD3, respectively. Chemotaxis tested the extracts’ capacity to induce HIMEC migration, and the contribution of individual angiogenic factors was assessed by antibody blockade.

Control and IBD colonic mucosa was immunostained for the endothelial antigen CD31, and vessels quantified by morphometry (vessel density/field). Microvessel αvβ3 expression in vivo was verified by confocal microscopy, and in human intestinal microvascular endothelial cells (HIMEC) cultures stimulated by bFGF, VEGF and TNF-α by flow cytometry. Pro-angiogenic activity of mucosal extracts was tested in vivo using a chick embryo assay, and in vitro by induction of HIMEC migration (cells/field).

Microvessel density was significantly (p < 0.05) higher in CD (25 ± 6) and UC (24 ± 4) compared to control mucosa (15 ± 3). αvβ3 was only sporadically detected in normal mucosa, but was ubiquitously expressed on IBD vessels as revealed by co-localization with CD31, bFGF and TNF-α, but not VEGF, markedly upregulated αvβ3 expression in HIMEC. IBD extracts visibly increased vascularization in chick embryos to the same extent than VEGF. Both CD (65 ± 12) and UC (62 ± 10) extracts dose-dependently induced HIMEC migration to a sig-
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significantly (p < 0.05) greater degree than controls (25 ± 6), a phenomenon primarily dependent on IL-8, and less on bFGF or VEGF, as shown by antibody neutralization studies.

Compared to controls (22 ± 6 pg/ml), IL-8 levels in IBD extracts were remarkably (p < 0.01) higher in UC (698 ± 180) and CD (456 ± 170), and the same was true for bFGF (UC: 1450 ± 60, CD: 1376 ± 55, control: 802 ± 33). VEGF showed a 5-fold increased in IBD compared to normal mucosa. Unstimulated HIF produced negligible amounts of IL-8, but low and high amounts of bFGF and VEGF, respectively. After TNF-α stimulation, production of IL-8 and VEGF increased dramatically, while that of bFGF remained unchanged. In contrast, LPMC spontaneously produced large amounts of all three mediators, and these increased with LPS and anti-CD3 stimulation for IL-8, while only LPS enhanced bFGF and VEGF. Both CD and UC extracts induced a 4-fold increase in HIMEC migration, which was inhibited primarily by blocking IL-8, but less bFGF and VEGF.

Based on morphological and functional evidence of pro-angiogenic activity in CD and UC mucosa, it is concluded that IBD microvasculature undergoes an intense process of neoangiogenesis. This suggests that neoangiogenesis is a vital component of IBD pathogenesis, and provides the material and conceptual bases for considering anti-angiogenic strategies for IBD therapy, as currently being tested in other autoimmune disorders.

References

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Acknowledgements

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