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Differential Angiogenic Regulation of Experimental Colitis

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Inflammatory bowel diseases (IBDs) are chronic inflammatory disorders of the intestinal tract with unknown multifactorial etiology that, among other things, result in alteration and dysfunction of the intestinal microvasculature. Clinical observations of increased colon microvascular density during IBD have been made. However, there have been no reports investigating the physiological or pathological importance of angiogenic stimulation during the development of intestinal inflammation. Here we report that the dextran sodium sulfate and $CD4^+CD45RB^{high}$ T-cell transfer models of colitis stimulate angiogenesis that results in increased blood vessel density concomitant with increased histopathology, suggesting that the neovasculature contributes to tissue damage during colitis. We also show that leukocyte infiltration is an obligatory requirement for the stimulation of angiogenesis. The angiogenic response during experimental colitis was differentially regulated in that the production of various angiogenic mediators was diverse between the two models with only a small group of molecules being similarly controlled. Importantly, treatment with the anti-angiogenic agent thalidomide or ATN-161 significantly reduced angiogenic activity and associated tissue histopathology during experimental colitis. Our findings identify a direct pathological link between angiogenesis and the development of experimental colitis, representing a novel therapeutic target for IBD. ***(Am J Pathol 2006, 169:2014–2030; DOI: 10.2353/ajpath.2006.051021)***

The inflammatory bowel diseases (IBDs), including Crohn's disease and ulcerative colitis, are chronic inflammatory disorders of the intestinal tract that are thought to arise from a complex interaction among the environment, the immune system, and genetics of affected individuals.[1–5] Although the specific causes of IBD are not well understood, several hallmark pathological features have been defined. The pathology of IBD invariably involves a chronic inflammatory cycle characterized by leukocyte infiltration, intestinal mucosa damage, ulceration, and regeneration. Recent clinical studies of active IBD have alluded to an increase in angiogenesis.[6,7] These studies suggest that stimulation of angiogenesis may play an important pathophysiological role in establishing and sustaining tissue inflammation, thereby playing an integral role in IBD pathology. Specifically, Spalinger and colleagues[6] have reported increased vessel density with Doppler ultrasonography during active Crohn's disease that is not enhanced in unaffected tissue or during disease remission. Moreover, a report by Fishman and colleagues[8] has demonstrated long-term remission of an individual with active Crohn's disease using the anti-angiogenic agent thalidomide. However, the pathological nature and importance of increased angiogenesis during IBD or experimental colitis is not known.

Angiogenic cytokine production has been reported to be elevated during IBD, possibly governing increased neovascularization during disease. Serum from IBD patients was reported to contain elevated concentrations of vascular endothelial growth factor (VEGF)-A during active disease versus remission.[9–11] A recent report by Konno and colleagues[12] has also shown elevated local tissue concentrations of VEGF-A in IBD specimens that may be more relevant in relation to IBD pathology, as it has been shown that local microenvironment tissue levels of VEGF-A are key in determining normal versus pathological angiogenesis.[12,13] Moreover, studies have also

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shown increases in serum levels of basic fibroblast growth factor (b-FGF) and transforming growth factor (TGF)-β in patients with active ulcerative colitis (UC) or Crohn's disease (CD).[10,14] Together, these reports clearly demonstrate that angiogenic cytokines are increased in patients with active IBD. However, no information is available regarding distinct expression profiles of pro- versus anti-angiogenic molecules during colitis.

Increased leukocyte infiltration is a hallmark feature of IBD and experimental colitis, with T cells, monocytes, and neutrophils contributing to disease initiation and subsequent tissue damage.[15–18] In addition to their role in the inflammatory response, leukocytes may also regulate angiogenic activity. Several studies have shown that various leukocyte types produce diverse angiogenic factors and can also modify extracellular matrix thereby promoting neovascularization.[19–21] Neutrophils have been reported to be significant sources of cytokines, such as interleukin (IL)-8 and VEGF, as well as matrix metalloproteinases (MMP-2 and -9) that are released at the site of inflammation to regulate angiogenesis.[22,23] Monocytes and macrophages also have corresponding roles in angiogenesis as sources of cytokines such as VEGF, FGFs, interferon-γ, tumor necrosis factor (TNF)-α, and many others.[24–26] It is widely accepted that T cells regulate IBD pathogenesis through the production of proinflammatory cytokines, such as Th1 cytokines, that are primarily proangiogenic concomitant with reduced production of regulatory cytokines, such as Th2 cytokines, that may have angiostatic or anti-angiogenic effects.[27–29] Therefore, infiltrated T cells could elicit diverse angiogenic regulation through the production of a wide array of angiogenic molecules including Th1 and Th2 cytokines and chemokines. Thus it is very likely that leukocyte infiltration with concomitant production of angiogenic mediators facilitates neovascularization and chronic inflammation observed in IBD.

Here we examine the hypothesis that increased angiogenic activity plays an important pathological role in two different models of experimental colitis. Data contained herein demonstrate that blood vessel density increases during colitis and strongly correlates with pathological scores of affected tissue. Moreover, we report that angiogenic stimulation in both models shows some similarities, as well as distinct differences, in angiogenic gene and protein profile expression, demonstrating a diverse angiogenic response. Data are also presented that demonstrate an essential requirement of leukocyte infiltration for angiogenic stimulation during colitis. Last, we report that anti-angiogenic intervention significantly attenuates leukocyte infiltration and tissue damage associated with experimental colitis, indicating that increased angiogenesis during colitis plays an important pathological role during disease. Together, these data provide compelling evidence for an important pathophysiological role of increased angiogenic activity during experimental colitis and highlight the potential for anti-angiogenic intervention as a novel therapeutic target for IBD.

Materials and Methods

Animals

Mice used for this study were bred and housed at the Association for Assessment and Accreditation of Laboratory Animal Care, international-accredited Louisiana State University Health Sciences Center–Shreveport animal resource facility and maintained according to the National Research Council's Guide for Care and Use of Laboratory Animals. CD18-null$^{-/-}$ (*Itgb2^{tm2Bay}*) C57BL/6J and wild-type C57BL/6J mice were bred in-house. Male Rag-1$^{-/-}$-null (*Rag1^{tm1Mom}*) C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME).

3% Dextran Sodium Sulfate (DSS) and CD4$^+$CD45RB^{high} T-Cell Transfer Models of Experimental Colitis

The 3% DSS and CD4$^+$CD45RB^{high} T-cell transfer models of colitis were performed as previously reported.[16,30] Ten- to 12-week-old wild-type or CD18$^{-/-}$-null male C57BL/6J mice were administered 3% DSS (TDB Consultancy AB, Uppsala, Sweden) in their drinking water for 6 days. Control cohorts were given regular drinking water. Animals were monitored for symptoms of disease progression as previously reported and sacrificed on the 7th day for analysis of various parameters described below. The 3% DSS cyclic colitis model was performed with slight modifications as previously reported.[31] In brief, 3% DSS was administered for 5 days (on) and then switched to regular water for another 5 days (off). This cycle was repeated three times, and mice were sacrificed at the end of the third off cycle.

CD4$^+$CD45RB^{high} T-cell-dependent colitis was induced in male Rag-1-null mice between 10 and 12 weeks of age using CD4$^+$ T cells from the spleens of donor female C57/B6J mice as previously reported.[16,32] In brief, CD4$^+$ T cells were enriched using the MACS system from Miltenyi Biotech for negative selection by magnetic cell sorting. Cells were then labeled with anti-fluorescein isothiocyanate (FITC) microbeads, and unlabeled cells were separated on a depletion column (column type CS; Miltenyi Biotech, Auburn, CA). Enriched CD4$^+$ T cells were labeled with biotin-conjugated anti-CD4 monoclonal antibody followed with streptavidin-670 and phycoerythrin-conjugated anti-CD45RB monoclonal antibody (PharMingen, La Jolla, CA) and fractioned into CD4$^+$CD45RB^{high} and CD4$^+$CD45RB^{low} cell populations by two-color sorting on a FACS Vantage (Becton, Dickinson and Company, Mountain View, CA). The CD45RB^{high} population was defined as the brightest 40% and that of the CD45RB^{low} were the dimmest 15%. Next, 5×10^5 CD4$^+$CD45RB^{high} or CD4$^+$CD45RB^{low} cells were injected intraperitoneally in 500 μl of phosphate-buffered saline (PBS). Animals were monitored throughout 8 weeks for the development of experimental colitis and sacrificed at the end of week 8 for analysis of the various parameters described below.