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Mouse strain influences angiogenic response to dextran sodium sulfate–induced colitis

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ABSTRACT

Background: Angiogenesis is a known pathologic factor in chronic inflammatory diseases. Regarding the murine dextran sodium sulfate (DSS) colitis model, different mouse strains produce variable clinical and inflammatory responses. We hypothesize that DSS colitis applied to diverse mouse strains will similarly elevate colonic microvessel density in parallel with inflammation, but will do so with different angiogenic profiles.

Materials and methods: We induced DSS colitis in 129S2/SvPas, BALB/c, and C57BL/6 mice, then performed histologic and molecular analysis at day 7 to evaluate colonic inflammation and angiogenesis.

Results: Inflammation and microvessel density were similarly increased in DSS groups. The C57BL/6 cohort mounted a more severe colitis with 25% weight loss and greater colonic ulceration. Gene expression of angiogenic factors at baseline and in colitis groups were widely variable among strains. BALB/c mice exhibited higher angiogenic gene expression in control and DSS groups compared with other strains, specifically platelet-derived growth factor, angiopoietin-1, angiopoietin-1 (Ang-2), vascular endothelial growth factor receptor, and PDGF receptor. When evaluating the effect of DSS relative to controls, BALB/c mice were not significantly affected. 129S2/SvPas mice exhibited broad suppression of growth factors, significantly platelet-derived growth factor, Ang-2, and PDGF receptor. In contrast, C57BL/6 mice displayed increased gene expression, especially for angiopoietin-1 and Ang-2.

Conclusions: Genetic heterogeneity influences the angiogenic profile elicited by DSS colitis. We demonstrate that within a model of murine colitis, mouse strain significantly affects inflammation-associated angiogenesis. These results may impact strain selection when using a colitis model focusing on inflammation and angiogenesis. Future studies to further define the angiogenesis pathway and potentially alter the disease course with targeted antiangiogenics are warranted.

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1. Introduction

Angiogenesis is a well-researched component in neoplastic disease and is increasingly significant in many chronic inflammatory conditions [1,2]. Inflammatory bowel disease (IBD) results from a multifactorial process with recent attention gained by inflammation-associated angiogenesis. Patients with IBD have increased vascular density detectible by Doppler ultrasound in bowel segments with active disease [3]. Severe IBD is often managed with infliximab, an antitumor necrosis factor α known to lessen disease severity and inflammation. An unpredicted effect of infliximab in responding patients is decreased mucosal angiogenesis [4]. The diseased intestine may experience pathologic vascular remodeling either by initiating or exacerbating the inflammatory disease [5].

The most critical mediator of angiogenesis and vascular remodeling is vascular endothelial growth factor (VEGF), but the process requires additional growth factors working in concert such as angiopoietin-1 (Ang-1) and platelet-derived growth factor (PDGF) to promote vascular maturation and stabilize as well as angiopoietin-1-2 (Ang-2) that destabilizes endothelium in preparation for remodeling [6,7]. Applying this mechanistic knowledge to disease contributed to the development of VEGF-targeted antiangiogenic therapies, most notably in colorectal cancer and age-related macular degeneration [2,8–10]. Human IBD exhibits increased gut mucosal vascular density at the histologic level and elevations in local and systemic VEGF [11–13]. Similar findings are supported in murine models of IBD, again demonstrating elevated inflammation-associated angiogenesis and VEGF [13–15].

Variability in the angiogenic profile exists among different models of murine IBD. For example, between the dextran sodium sulfate (DSS) and $CD4^+CD45RB^{high}$ T cell transfer models of colitis only about 30% of angiogenic factors were similarly up- or down-regulated, whereas VEGF was suppressed in the DSS model and elevated in the T cell transfer model [14]. This demonstrates that not only the absolute gene expression may differ between models but also can demonstrate the angiogenic profile. Variability in response to experimental conditions is also seen within a single model of experimental colitis (DSS). When applied to different mouse strains, DSS produces a wide range of results regarding degree of weight loss, location of colonic disease, extent of ulceration, severity of inflammation, and cytokine profile [16–19]. No studies have explored the potential influence of genetic heterogeneity as it pertains to angiogenesis in experimental IBD. In the commonly used model of DSS-induced colitis, we suspect that the angiogenic profile may differ among mouse strains, similar to the diverse inflammatory response among strains previously noted. This concept is vital to the investigation of inflammation-associated angiogenesis in experimental IBD but is currently unexplored in the literature. We hypothesize that DSS colitis applied to multiple mouse strains (129S2/SvPas, BALB/c, and C57BL/6) will similarly elevate colonic microvessel density (MVD) in parallel with inflammation, but will do so with diverse histopathology and variable angiogenic profiles. Murine strain selection was restricted to those commonly used in this model of colitis with a predilection for left-sided colonic disease and free of significant comorbidities such as susceptibility to immunologic diseases including diabetes mellitus in nonobese diabetic mice [17,19,20].

2. Methods

2.1. Murine colitis model

Male mice were used to limit genetic variability based on gender and because of their enhanced inflammation in the DSS model [17,21]. Three different strains aged 5–7 wk (129S2/SvPas, BALB/c, and C57BL/6) were acquired from Charles River Laboratories or Harlan Laboratories, Indianapolis, IN and allowed to acclimate in barrier housing for 1–2 wk with bottles as the sole source of water. Our standard 1 wk period for acclimation was increased for the 5-wk-old mice to allow closer age comparisons among strains. Drinking water was supplemented with 2% DSS *ad libitum* for 7 d in the colitis groups, whereas wild type (WT) mice received regular water ($n = 4$–6 per group). Clinical signs and symptoms of colitis were monitored daily through necropsy at day 7. All experiments were performed in accordance with our Institutional Animal Care and Use Committee (IACUC No. 2D04026).

2.2. Histopathologic colitis score

Fixed tissues were preserved in 4% paraformaldehyde followed by serial alcohol dehydrations. Colons were stained with hematoxylin and eosin, then evaluated by light microscopy to score the four consecutive high-powered fields (HPFs) proximal to the anorectal junction at ×40 magnification. An accepted colitis scoring method [22,23] was applied by two blinded observers to obtain a cumulative score (range 0–18) based on following categories: (1) percent area involved, (2) ulceration, (3) crypt loss, (4) edema, and (5) immune cell infiltration.

2.3. Immunohistochemistry

Tissue sections were deparaffinized and rehydrated. A citrate-based epitope enhancement with Antigen Retrieval Solution (DAKO, Carpinteria, CA) was applied followed by Protein Blocker Solution (DAKO). After incubation with MECA-32 primary monoclonal antibody (1:10; Developmental Studies Hybridoma Bank, Iowa City, IA) then Alexa 488–tagged fluorescent secondary antibody (1:200; Invitrogen, Carlsbad, CA), slides were mounted using 4′,6-diamidino-2-phenylindole (DAPI) Vectashield (Vector Laboratories, Burlingame, CA).

2.4. Microvessel density

After endothelium immunohistochemical stain (MECA-32), specimens were microphotographed for 10 consecutive, distal fields at ×200 magnification using fluorescent microscopy (Nikon 90i; Nikon Instruments, Inc, Melville, NY). Average quantitative analysis of mucosal MVD per HPF (square micrometer) was performed using NIS-Advanced Elements software (Nikon Instruments, Inc).