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Intussusceptive remodeling of vascular branch angles in chemically-induced murine colitis ☆

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ABSTRACT

Intussusceptive angiogenesis is a developmental process linked to both blood vessel replication and remodeling in development. To investigate the prediction that the process of intussusceptive angiogenesis is associated with vessel angle remodeling in adult mice, we systematically evaluated corrosion casts of the mucosal plexus in mice with trinitrobenzesulfonic acid (TNBS)-induced and dextran sodium sulfate (DSS)-induced colitis. The mice demonstrated a significant decrease in vessel angles in both TNBS-induced and DSS-induced colitis within 4 weeks of the onset of colitis ($p < .001$). Corrosion casts 28–30 days after DSS treatment were studied for a variety of detailed morphometric changes. The vessel diameter and interbranch distance were significantly increased in the descending colon ($p < .05$). Also consistent with vessel growth, intervascular distance was decreased in the descending colon ($p < .05$). In contrast, no statistically significant morphometric changes were noted in the ascending colon. The morphometry of the corrosion casts also demonstrated 1) a similar orientation of the remodeled angles within the XY coordinate plane of the mucosal plexus, and 2) alternating periodicity of remodeled and unremodeled vessel angles. We conclude that inflammation-associated intussusceptive angiogenesis in adult mice is associated with vessel angle remodeling. Further, the morphometry of the vessel angles suggests the influence of blood flow on the location and orientation of remodeled vessels.

Introduction

Blood vessel bifurcations are an essential structural feature of all vascular networks. In tree-like branching patterns, blood flow travels through the parent vessel into two smaller diameter daughter vessels. The ubiquity of this fluid transport system in nature has led to considerable theoretical work attempting to produce a theory of universal bifurcation design (LaBarbera, 1990). Many of these theories are based on the premise that vessel geometry is organized on the principle of economy. Most notably, "Murray's law" is the hypothesis that vascular architecture is driven by functional optimization; namely, vascular design reflects the minimal amount of energy necessary to maintain and circulate the blood (Murray, 1926). Although "Murray's law" is remarkably accurate in predicting the branching diameters of large vessels, it is less accurate in the microcirculation. Particularly in non-tree branching patterns, such as plexuses and arcades, vessel bifurcations demonstrate wide geometric variation that cannot be explained by simple cost functions.

An intriguing non-tree branching vessel pattern is observed in the colonic mucosal plexus (Miele et al., 2009; Tsuda et al., 2009; Turhan et al., 2007). The mucosal plexus is a quasi-polygonal planar network that encircles the colonic crypts. In normal circumstances, the mucosal plexus supplies nutrients to the highly metabolic digestive epithelium. In inflammatory conditions such as acute and chronic colitis, the mucosal plexus undergoes active intussusceptive angiogenesis resulting in a dramatic increase in mucosal vascularity (Konerding et al., 2010).

Intussusceptive angiogenesis is a well-characterized morphogenetic process in cancer, inflammation and regeneration in which a single vessel is split into two lumens (Konerding et al., 2010, 2012). A distinguishing anatomic feature of intussusceptive angiogenesis is the intussusceptive pillar. The intussusceptive pillar is a 1–5 μm (Burri et al., 2004) transluminal tissue bridge that spans the vessel lumen; its small size typically requires corrosion casting and scanning electron microscopy (SEM) for visualization (Lee et al., 2010, 2011). Physical expansion or growth of the pillar along the vessel axis divides the lumen resulting in vascular duplication. In addition to vessel division, the intussusceptive pillar can rapidly change the geometry of

Abbreviations: 2D, 2-dimensional; 3D, 3-dimensional; CFSE, 5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester; DSS, dextran sodium sulfate; FITC, isothiocyanate; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TNCB, 2,4,6-trinitrochlorobenzene.

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the affected vessel branch path (Djonov et al., 2002; Kurz et al., 2003). Whether the intussusceptive pillar contributes to vessel angle remodeling in murine colitis is unknown.

In this report, we investigated the hypothesis that intussusceptive angiogenesis, previously observed in murine colitis (Konerding et al., 2010), was also associated with branch angle remodeling. Our results suggest that pillar formation in chemically-induced colitis leads to both intussusceptive angiogenesis and branch angle remodeling.

Methods

Mice

Male Balb/c (N = 183) or C57B/6 (N = 244) mice (Jackson Laboratory, Bar Harbor, ME), 25–33 g, were used in all experiments. The care of the animals was consistent with the guidelines of the American Association for Accreditation of Laboratory Animal Care (Bethesda, MD).

Trinitrobenzesulfonic acid (TNBS) colitis

Because of strain-specific sensitivity to TNBS (Elson et al., 1996), BALB/c mice were used for TNBS experiments. The mouse abdomen was sheared and cleansed with water; 24 h later, 36 μl of a 2.5% trinitrochlorobenzene (TNCB) (ChemArt, Germany) solution was sprayed onto a 1.5 cm diameter circular PhastTransfer Filter Paper (Pharmacia, Uppsala, Sweden) in a 4:1 acetone:olive oil mixture. The antigen soaked filter paper was applied to sheared abdomen with Tegaderm (3 M, St. Paul, MN) for 24 h. On day 0, and at 7 day intervals, 36 μl of TNBS was administered intrarectally. The mice were assessed daily for clinical signs and total body weight.

Dextran sodium sulfate (DSS) colitis

In C57/B6 mice, the dextran sodium sulfate (DSS) (TdB Consultancy AB, Uppsala, Sweden) model of colitis was similar to that described previously (Okayasu et al., 1990). Briefly, DSS was freshly prepared and added daily to the mice drinking water at a final concentration of 5%. The mice were assessed daily for clinical signs and total body weight. The DSS treatment was continued for 5 days then changed to water for the remainder of the experimental period.

Clinical assessment of colitis

Clinical parameters including total body weight were assessed daily (Ravnic et al., 2007a). Activity level and fur ruffling were scored daily on a 0 (normal) to 2 (severe) scale. A change in body weight was also assigned a clinical colitis score: >5% of the baseline weight = 1 point; >10% = 2 points.

Scanning electron microscopy

Corrosion casting and scanning electron microscopy (SEM) were performed in both TNBS (N = 32) and DSS (N = 64) mice. After systemic heparinization, PBS perfusion and intravascular fixation with 2.5% buffered glutaraldehyde, the systemic circulation was perfused with 10–20 ml of Mercox (SPI, West Chester, PA) diluted with 20% methyl methacrylate monomers (Aldrich Chemical, Milwaukee, WI) as described previously (Ravnic et al., 2006). After complete polymerization, the tissues were harvested and macerated in 5% potassium hydroxide followed by drying and mounting for scanning electron microscopy. The microvascular corrosion casts were imaged after coating with gold in an argon atmosphere with a Philips ESEM XL30 scanning electron microscope (Eindhoven, Netherlands). In some mice, stereo-pair images were obtained using a tilt angle of 6°. The quality of the filling of the corrosion casts was also checked by comparisons with the vascular densities in semithin light microscopic sections stained with methylene blue. The corrosion casts chosen for morphometric analysis demonstrated the filling of the whole capillary bed from artery to vein without the evidence of extravasation or pressure distension.

Measurement of branch angles and pillars

Similar to previous studies emphasizing angle geometry (Thomas et al., 2005), the method of branch angle morphometry was designed to be sensitive to variation at the apex of the bifurcation. In layered images, maximally-sized spheres were inscribed in each vessel at the bifurcation (Fig. 1). Sequential spheres within each vessel were inscribed so that the surface of the sphere intersected the centerpoint of the preceding sphere. The centerline track of the first two spheres was used to define the vessel coordinates. In cases with unusual tortuosity, in which the centerline track did not project over the vessel lumen, a third or fourth sphere centerpoint was used. The bifurcation angle was measured as the angle between centerline tracks using digital morphometry software. To minimize bias, the vessel angles were measured by observers blinded to the experimental conditions. The mean value of the observers was recorded. Representative stereopairs of vascular casts were analyzed for the assessment of bifurcations showing features of intussusceptive angiogenesis. Tiny holes and small capillary loops with a diameter between 1 and 5 μm at the bifurcation angles were identified as pillars on each image area and were assessed related to all bifurcations.

Morphometric measurements

The measurements of vessel diameters (Ravnic et al., 2007b) as well as interbranch (Ravnic et al., 2005) and intervascular (Malkusch et al., 1995) distances were performed as previously described.

Statistical analysis

The statistical analysis was based on measurements in at least three different mice. The unpaired Student's *t* test for the samples of unequal variances was used to calculate statistical significance. The data was expressed as mean ± one standard deviation. The significance level for the sample distribution was defined as $P < .01$.

Results

Intussusceptive angiogenesis in colitis

Two models of chemically-induced colitis (TNBS and DSS) were studied for 30 days. The clinical signs of colitis—such as ruffled fur, decreased activity and weight loss—were commonly observed 4 to 7 days after the onset of inflammation (Fig. 2A). Similarly, the highest mortality was observed within the first 5 days (Fig. 2B). Coincident with the resolution of the clinically apparent colitis, intussusceptive pillars were observed at bifurcations within the mucosal plexus (Fig. 2C). Visible as "holes" within the corrosion casts (Figs. 2Ca, b), pillars were the structural feature that signaled the onset of intussusceptive angiogenesis (Konerding et al., 2010). Intussusceptive pillars at vessel bifurcations were significantly more prevalent in DSS-colitis mice than in control mice (13% versus 5%; $p < .001$); nonetheless, the presence of pillars in control mice suggested that intussusceptive processes (angiogenesis and angle remodeling) participate in morphostasis; that is, routine mucosal maintenance and repair.

Vessel angle remodeling in colitis

To investigate the prediction that the process of intussusceptive angiogenesis is associated with vessel angle remodeling, we systematically evaluated corrosion casts of the mucosal plexus after chemically-induced