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CXCL5 is required for angiogenesis, but not structural adaptation after small bowel resection

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ABSTRACT

Purpose: Intestinal adaptation is the compensatory response to massive small bowel resection (SBR) and characterized by lengthening of villi and deepening of crypts, resulting in increased mucosal surface area. Previous studies have demonstrated increased villus capillary blood vessel density after SBR, suggesting a role for angiogenesis in the development of resection-induced adaptation. Since we have previously shown enhanced expression of the proangiogenic chemokine CXCL5 after SBR, the purpose of this study was to determine the effect of disrupted CXCL5 expression on intestinal adaptation.

Methods: CXCL5 knockout (KO) and C57BL/6 wild type (WT) mice were subjected to either a 50% proximal SBR or sham operation. Ileal tissue was harvested on postoperative day 7. To assess for adaptation, villus height and crypt depth were measured. Submucosal capillary density was measured by CD31 immunohistochemistry.

Results: Both CXCL5-KO and WT mice demonstrated normal structural features of adaptation. Submucosal capillary density increased in the WT but not in the KO mice following SBR.

Conclusion: CXCL5 is required for increased intestinal angiogenesis during resection-induced adaptation. Since adaptive villus growth occurs despite impaired CXCL5 expression and enhanced angiogenesis, this suggests that the growth of new blood vessels is not needed for resection-induced mucosal surface area expansion following massive SBR.

Short gut syndrome results from substantial intestinal loss and is a condition of high morbidity and mortality within the pediatric population. Following massive small bowel resection (SBR), intestinal adaptation is a critical, compensatory response in both humans and animal models that allows for adequate absorption of enteral nutrition despite significant loss of bowel length [1–3]. This phenomenon is characterized by significant increases in villus height and crypt depth, in part owing to increased enterocyte proliferation, and resulting in increased absorptive mucosal surface area to compensate for the attenuated bowel length [1].

Angiogenesis is the growth of new blood vessels and is known to play a significant role in general states of cellular proliferation [4,5]. Within the intestine, supplementation of proangiogenic growth factors has been shown to enhance intestinal mucosal growth [6]. Alternatively, inhibition of vascular endothelial growth factor (VEGF) resulted in a decreased adaptive response following intestinal loss [7]. We have previously reported increased villus capillary density during the intestinal adaptation response to massive SBR [8]. This increased villus capillary density in the intestine is preceded by an increase in the intestinal gene expression of proangiogenic chemokine ligand 5 (CXCL5) [8,9].

CXL5 belongs to the CXC chemokine family of molecules that bind the CXCR2 receptor and serve as neutrophil chemoattractants and promoters of neovascularization [10–12]. CXCL5 has been demonstrated to play a role in neutrophil homeostasis at mucosal sites, including the intestine [13]. In patients with inflammatory bowel disease, CXCL5 has been shown to be overexpressed in the intestinal epithelial cells [14,15]. CXCL5 expression is enhanced in murine models of colitis and inhibition of CXCL5 attenuates disease severity [16,17]. As well, CXCL5 expression has been shown to be upregulated in human necrotizing enterocolitis (NEC) intestinal tissue [18]. In murine neonatal models of NEC, CXCL5 has been shown to recruit macrophages to the gastrointestinal tract during inflammatory mucosal injury [18].

Since we have previously shown increased intestinal expression of CXCL5 after SBR [8,9], the purpose of the present study was to determine the effects of absent CXCL5 expression on structural features of intestinal adaptation as well as angiogenic responses to massive SBR.

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1. Materials and methods

1.1. Experimental design

A protocol for this study was approved by the Washington University Animal Studies Committee (Protocol #20100103) and in accordance with the National Institute of Health laboratory animal care and use guidelines. Four experimental groups were studied: wild type (WT) mice that underwent sham operation (n = 9) or 50% proximal SBR (n = 6), and CXCL5 knockout (KO) mice that underwent sham operation (n = 10) or 50% proximal SBR (n = 13). CXCL5 gene deletion was confirmed via RT-PCR of CXCL5 mRNA within the small intestine. Ileal tissue was harvested in all mouse groups on postoperative day 7. To assess for adaptation, villus height and crypt depth were measured via hematoxylin and eosin (H&E)-stained histology. In addition, submucosal capillary density was measured by CD31 immunohistochemistry.

1.2. Animals

CXCL5 KO mice on a C57BL/6 background were generously provided by Junjie Mei and Scott Worthen, Children's Hospital of Philadelphia (Philadelphia, PA) [19]. Nonmutant C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) were used as WT controls. Male and female mice aged 7–15 weeks were used in this study with a weight range of 23.5 to 29.5 g (WT) and 18.0 to 28.0 g (KO). Mice were kept on a 12-hour light–dark schedule and were housed in a standard facility. The mice were given a liquid rodent diet (Micro-Stabilized Rodent Liquid Diet LD101; Purina Mills, St. Louis, MO) 1 day prior to surgery.

1.3. Operative technique

Mice underwent 50% proximal SBR or sham operation (transection and reanastomosis only) as previously described [1]. Briefly, mice that underwent SBR had transection of the bowel at a point 12 cm proximal to the ileal–cecal junction and also at a point 1 to 2 cm distal to the ligament of Treitz. The mesentery was ligated and the intervening bowel was removed. Intestinal continuity was restored with an end-to-end anastomosis using 9-0 monofilament suture. In mice undergoing sham operation the bowel was transected at a point 12 cm proximal to the ileal–cecal junction and intestinal continuity was restored with an end-to-end reanastomosis. Following the operation, mice were provided free access to water for the first 24 hours and then given a liquid rodent diet until sacrifice.

1.4. Tissue harvest

On the seventh postoperative day, the mice were anesthetized with a subcutaneous injection of ketamine, xylazine, and acepromazine (4:1:1). A midline laparotomy was performed and the small bowel was flushed with ice-cold phosphate-buffered saline and excised. The first 1 cm segment of bowel distal to the anastomosis was discarded. The next 2 cm segment of bowel was fixed in 10% neutral-buffered formalin for histology. Following tissue harvest, the animal was sacrificed via cervical dislocation.

1.5. RT-PCR confirmation of disrupted CXCL5 mRNA expression in enterocytes

Total RNA was extracted from ileal tissue following the manufacturer's protocol for the RNAqueous kit (Ambion, Austin, TX) and total RNA concentration determined spectrophotometrically. Quality of obtained RNA was evaluated using the Bio-Rad Experion System with an RNA StdSens Chip and reagents (Bio-Rad Laboratories, Richmond, CA). A TaqMan RNA-to Ct 1-Step kit (Applied Biosystems, Foster City, CA) was used per the manufacturer's protocol to determine relative gene expression directly from the isolated RNA. Equal amounts of RNA were used for real-time PCR with B-actin as endogenous control and a standard whole bowel sample used as calibrator. CXCL5 gene

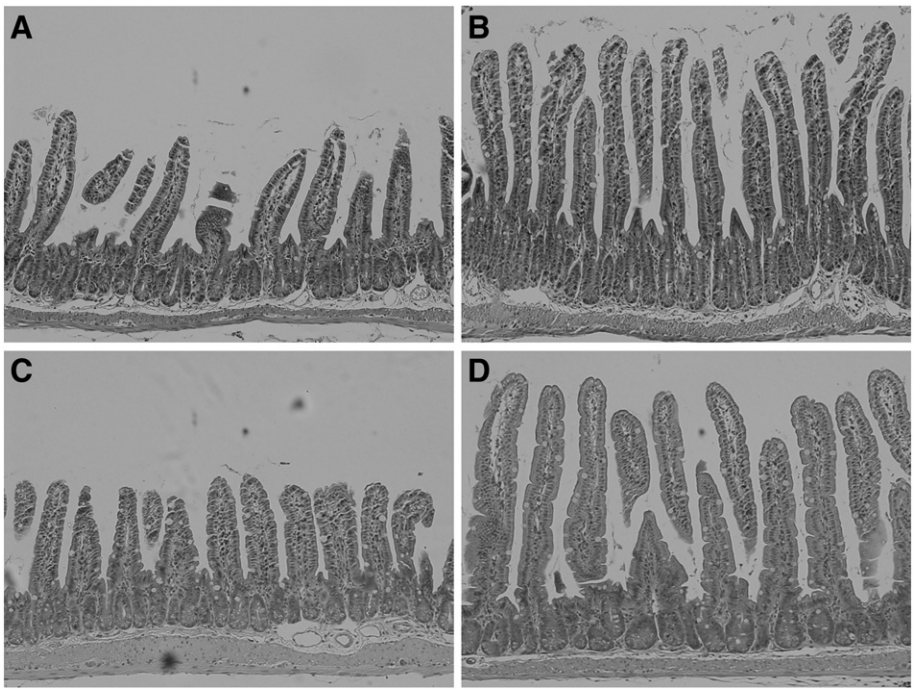

Fig. 1. Adaptation occurs normally after 50% proximal small bowel resection (SBR) in both wild-type (WT) and CXCL5 knockout (KO) mice. Hematoxylin and eosin (H&E)-stained section of mouse ileum (magnification 10×)—A: WT sham operation. B: WT 50% proximal small bowel resection (SBR). C: CXCL5-KO sham operation. D: KO 50% proximal SBR with complete villus and crypt adaptive response.