Epimorphin$^{-/-}$ mice are protected, in part, from acute colitis via decreased interleukin 6 signaling

ANISA SHAKER, MATTHEW GARGUS, JULIE FINK, JANA BINKLEY, ISRA DARWECH, ELZBIETA SWIETLICKI, MARC S. LEVIN, and DEBORAH C. RUBIN

LOS ANGELES, CA; ST. LOUIS, MO

Epimorphin (Epim), a member of the syntaxin family of membrane-bound, intracellular vesicle-docking proteins, is expressed in intestinal myofibroblasts and macrophages. We demonstrated previously that *Epimorphin*$^{-/-}$ (*Epim*$^{-/-}$) mice are protected, in part, from dextran sodium sulfate (DSS)-induced colitis. Although interleukin (IL)-6/p-Stat3 signaling has been implicated in the pathogenesis of colitis, the myofibroblast contribution to IL-6 signaling in colitis remains unexplored. Our aim was to investigate the IL-6 pathway in *Epim*$^{-/-}$ mice in the DSS colitis model. Whole colonic tissue, epithelium, and stroma of WT and congenic *Epim*$^{-/-}$ mice treated with 5% DSS for 7 days were analyzed for IL-6 and a downstream effector, p-Stat3, by immunostaining and immunoblot. Colonic myofibroblast and peritoneal macrophage IL-6 secretion were evaluated by enzyme-linked immunosorbent assay. IL-6 and p-Stat3 expression were decreased in *Epim*$^{-/-}$ vs WT colon. A relative increase in stromal vs epithelial p-Stat3 expression was observed in WT mice but not in *Epim*$^{-/-}$ mice. Epim deletion abrogates IL-6 secretion from colonic myofibroblasts treated with IL-1β and decreases IL-6 secretion from peritoneal macrophages in a subset of DSS-treated mice. Epim deletion inhibits IL-6 secretion most profoundly from colonic myofibroblasts. Distribution of Stat3 activation is altered in DSS-treated *Epim*$^{-/-}$ mice. Our findings support the notion that myofibroblasts modulate IL-6/p-Stat3 signaling in DSS-treated *Epim*$^{-/-}$ mice. (Translational Research 2014;164:70–83)

Abbreviations: α-SMA = α-smooth muscle actin; CD = Crohn's disease; DSS = dextran sodium sulfate; EDTA = ethylenediamine tetraacetic acid; ELISA = enzyme-linked immunosorbent assay; Epim = *Epimorphin (Epim)*$^{-/-}$; IBD = inflammatory bowel disease; Ig = immunoglobulin; IL = interleukin; ISEMF = intestinal subepithelial myofibroblast; PBS = phosphate-buffered saline; Stat3 = signal transducer and activator of transcription 3; UC = ulcerative colitis

Ulcerative colitis (UC) and Crohn's disease (CD) are complex, polygenic gastrointestinal inflammatory disorders that afflict millions of individuals in the United States and worldwide.[1] Accumulating evidence suggests that inflammatory bowel disease (IBD) develops, in part, as a consequence of a dysregulated immune response to environmental factors/gut microbiota in the genetically susceptible host.[1-4] Nontraditional innate immune cells, such as intestinal subepithelial myofibroblasts (ISEMFs), have also been implicated in the pathogenesis of IBD.

ISEMFs are mesenchymal cells located subjacent to the basement membrane, at the interface between the epithelium and lamina propria. ISEMFs participate in epithelial-mesenchymal crosstalk and in mediating the immune response via secretion of cytokines, growth factors, and inflammatory mediators.[5] An increase in myofibroblast number has been observed in inflamed UC and CD mucosa.[6] Myofibroblasts in inflamed tissue are

From the Divisions of Gastroenterology and Hepatology, Keck School of Medicine of USC, Los Angeles, CA; Divisions of Gastroenterology and Hepatology, Washington University School of Medicine, St. Louis, Mo; Department of Medicine, St. Louis Health Care System, St. Louis, Mo.

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Reprint requests: Anisa Shaker, MD, Keck School of Medicine, University of Southern California, 2011 Zonal Avenue, HMR 810, Los Angeles, CA 90089-0110; e-mail: ashaker@usc.edu.

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AT A GLANCE COMMENTARY

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Background

Inflammatory bowel disease (IBD) is, in part, a consequence of a dysregulated immune response in the genetically susceptible host. Although intestinal subepithelial myofibroblasts are likely participants in IBD pathogenesis, myofibroblast contributions to implicated signaling pathways remain unexplored. Our aim was to investigate myofibroblast contributions to the interleukin (IL)-6 pathway in a murine model of colitis.

Translational Significance

Our findings demonstrate the crucial role of the myofibroblast in IL-6/p-Stat3 signaling in colitis. An understanding of the stromal-mediated pathways in the pathogenesis of intestinal injury and inflammation is needed. Our findings are novel and have the potential for application to human disease.

characterized by altered proliferation and an increase in secretion of cytokines and extracellular matrix factors.[7] Tissue scarring and fibrosis observed in CD has been attributed to the presence of activated myofibroblasts.[7] As reviewed extensively by Pinchuk et al,[8] activated intestinal myofibroblasts produce proinflammatory mediators and molecules that may, ultimately, either downregulate or lead to chronic inflammation. Recent research has also provided insights into the immunosuppressive function of myofibroblasts. Human colonic myofibroblasts, for example, promote the expansion of a subset of regulatory T cells.[9]

These observations are consistent with the active role of myofibroblasts in the pathogenesis of colitis. To delineate the role of these cells further in the pathogenesis of intestinal disorders, we investigated the role of the unique mesenchymal protein epimorphin (Epim). Epimorphin (Epim) is a member of the syntaxin family of membrane-bound, intracellular vesicle-docking proteins known as target membrane SNAREs (t-SNARE) that mediate fusion of v-SNARE-expressing intracellular secretory vesicles with the plasma membrane.[10] In the gut, Epim is expressed in mesenchymal cells, including ISEMFs, and has a role in regulating intestinal morphogenesis.[11,12]

Investigations of the role of Epim in the adult intestine have shown that *Epim*$^{-/-}$ mice are protected, in part, from dextran sodium sulfate (DSS)-induced acute colitis,[13] a well-established, chemically induced model of IBD with similarities to UC[14,15] used frequently to investigate intestinal injury and repair mechanisms. The mechanisms of protection from DSS-induced injury in *Epim*$^{-/-}$ mice are, in part, a result of improved repair, mediated via an increase in crypt cell proliferation secondary to modulation of Bmp secretion.[13] *Epim*$^{-/-}$ mice also have a markedly reduced dysplastic tumor burden compared with WT mice in the azoxymethane/DSS murine model of colitis-associated cancer[16] mediated, in part, by a decrease in interleukin (IL)-6 secretion from LPS-stimulated intestinal mesenchymal cells.[16] Overall, these findings suggested that, in the gut, the effects of Epim are mediated via its functional homology to syntaxin 2, which in turn regulates secretion of signaling factors.[11] The IL-6/signal transducer and activator of transcription 3 (Stat3) signaling pathway has been implicated in the pathogenesis of colitis, with reports of elevated serum and tissue IL-6 in both IBD and animal models of colitis. IL-6-mediated induction of the transcription factor Stat3 induces lamina propria T-cell resistance against apoptosis, leading to T-cell accumulation and perpetuation of chronic inflammation.[17-19]

We were interested, therefore, in exploring the previously unexamined role of Epim deletion on the IL-6 pathway in the acute DSS colitis model. We evaluated IL-6 expression in DSS-treated colonic tissue of WT and congenic *Epim*$^{-/-}$ mice. IL-6 signaling was assessed further by evaluating expression of 1 of its major downstream effectors, p-Stat3, in whole colon, and in enriched colonic epithelium and stroma by immunostaining and immunoblot.

MATERIALS AND METHODS

Animals. Congenic *Epim*$^{-/-}$ (on a C57BL/6J background) and WT C57Bl/6J male littermates were treated for 7 days with a solution of filtered water containing 5% DSS (USB Corporation). Mice were housed at a Washington University School of Medicine barrier facility in a 12-hour light/dark cycle with complete access to food and water. The mice were fed a standard rodent chow diet (PicoLab 20; Purina). Age-matched 8- to 12-week-old *Epim*$^{-/-}$ and WT littermates were used in all experiments. All animal experimentation was approved by the Animal Studies Committee of Washington University School of Medicine, where the study was carried out.

DSS-induced colitis. Congenic *Epim*$^{-/-}$ mice (n = 27) and WT C57BL/6J mice (n = 18) age-matched male littermates were treated for 7 days with a solution of filtered water containing 5% DSS (USB Corporation) in water *ad libitum*. Daily weights, stool consistency,