Endothelial PAS Domain Protein 1 Activates the Inflammatory Response in the Intestinal Epithelium to Promote Colitis in Mice

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BACKGROUND & AIMS: Hypoxic inflammation (decreased oxygen tension at sites of inflammation) is a feature of inflammatory bowel disease (IBD). The hypoxia response is mediated by the transcription factors hypoxia-inducible factor (HIF) 1α and endothelial PAS domain protein 1 (EPAS1 or HIF2α), which are induced in intestinal tissues of patients with IBD. HIF1α limits intestinal barrier dysfunction, but the role of EPAS1 has not been assessed under conditions of hypoxic inflammation or in models of IBD. **METHODS:** Acute colitis was induced by administration of *Citrobacter rodentium* or dextran sulfate sodium (DSS) to transgenic hypoxia reporter mice (oxygen-dependent degradation–luciferase), mice with conditional overexpression of Epas1 (*Epas1*$^{LSL/LSL}$), mice with intestinal epithelium-specific deletion of *Epas1* (*Epas1*$^{\Delta IE}$), or wild-type littermates (controls). Colon tissues from these mice and from patients with ulcerative colitis or Crohn's disease were assessed by histologic and immunoblot analyses, immunohistochemistry, and quantitative polymerase chain reaction. **RESULTS:** Levels of hypoxia and EPAS1 were increased in colon tissues of mice after induction of colitis and patients with ulcerative colitis or Crohn's disease compared with controls. *Epas1*$^{\Delta IE}$ mice had attenuated colonic inflammation and were protected from DSS-induced colitis. Intestine-specific overexpression of EPAS1, but not HIF-1α, led to spontaneous colitis, increased susceptibility to induction of colitis by *C rodentium* or DSS, and reduced survival times compared with controls. Disruption of intestinal epithelial EPAS1 attenuated the inflammatory response after administration of DSS or *C rodentium*, and intestine-specific overexpression of EPAS1 increased this response. We found EPAS1 to be a positive regulator of tumor necrosis factor–α production by the intestinal epithelium. Blocking tumor necrosis factor–α completely reduced hypoxia-induced intestinal inflammation. **CONCLUSIONS: EPAS1 is a transcription factor that activates mediators of inflammation, such as tumor necrosis factor–α, in the intestinal epithelium and promotes development of colitis in mice.**

Keywords: Mouse Model; CD; UC; Oxygen.

Hypoxia is a well-conserved transcriptional stimuli leading to an adaptive increase in the cellular response to limited oxygen availability. Inflammatory foci are hypoxic and this focal hypoxia can drive the inflammatory cascade.[1,2] The significance of this bidirectional crosstalk and the mechanistic role of hypoxia in inflammation are currently unclear. Hypoxia-induced signal pathway is transcriptionally mediated by hypoxia-inducible factor (HIF).[3,4] In oxygen-rich conditions, prolyl hydroxylases (PHD) hydroxylate HIF leading to its degradation by the von Hippel-Lindau tumor suppressor protein coupled with the E3 ubiquitin ligase complex. Conversely, in conditions of low cellular O_2, HIF is not hydroxylated and stabilized. Two transcriptionally active HIFs have been identified, HIF-1α and endothelial PAS domain protein 1 (EPAS1, also referred to as HIF-2α). They share a 48% sequence identity and regulate overlapping and distinct sets of genes critical in the adaptation to hypoxic environments.

A robust activation of HIF-1α and EPAS1 is observed in the intestinal epithelium from inflammatory bowel disease (IBD) patients.[5] IBD is an inflammatory disease of the intestine, which is grouped into 2 major types, Crohn's disease (CD) and ulcerative colitis (UC). Although the etiologies of CD and UC are not known, the dysregulation in intestinal epithelial barrier function and the mucosal immune response are critical in the pathogenesis of IBD. Recent studies demonstrate that HIF-1α in intestinal epithelial cells is critical in maintaining the intestinal epithelial barrier after inflammation.[6–10] In addition, pharmacological activation of HIF signaling using PHD inhibitors is protective in mouse models of IBD.[11,12] Therefore, it is still unclear the mechanism by which hypoxia exacerbates the inflammatory cascade.[2,13] In this article, using novel mouse models of intestinal HIF signaling, we demonstrate that EPAS1 is highly activated in epithelial cells from IBD patients and mouse models of colitis. Genetic activation of EPAS1 in epithelial cells leads to an increase in epithelial-derived proinflammatory mediators, which are critical in the initiation and progression of IBD.

Abbreviations used in this paper: CD, Crohn's disease; DMOG, dimethyloxaloylglycine; DSS, dextran sulfate sodium; EPAS1, endothelial PAS domain protein 1; HIF, hypoxia-inducible factor; IBD, inflammatory bowel disease; IL, interleukin; MAZ, myc-associated zinc finger protein; NF-κB, nuclear factor κB; ODD, oxygen-dependent degradation; PHD, prolyl hydroxylase; TNF, tumor necrosis factor; UC, ulcerative colitis; WT, wild type.

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Materials and Methods

Animals and Treatment

Vhl$^{F/F}$, *Vhl*$^{\Delta IE}$, *Epas1*$^{F/F}$, *Epas1*$^{\Delta IE}$, *Vhl*$^{F/F}$/*Hif-1α*$^{F/F}$, *Vhl*$^{\Delta IE}$/*Hif-1α*$^{\Delta IE}$, *Vhl*$^{F/F}$/*Epas1*$^{F/F}$, *Vhl*$^{\Delta IE}$ /*Epas1*$^{\Delta IE}$, and the oxygen-dependent degradation (ODD)-luciferase mice were described previously.[13–17] LSL-HIF1 dPA and LSL-HIF2 dPA mice have been described previously.[18] The LSL-HIF1 dPA and LSL-HIF2 dPA mice were crossed with murine villin promoter-driven cre mice[19] to generate intestinal epithelial–specific HIF-1α and EPAS1-overexpressing mice (*Hif-1α*$^{LSL/LSL}$ and *Epas1*$^{LSL/LSL}$), respectively. *Hif-1α*$^{LSL/LSL}$ and *Epas1*$^{LSL/LSL}$ mice were crossed to each other to generate intestinal epithelial–specific HIF-1α and EPAS1-double–overexpressing mice (*Hif-1α*$^{LSL/+}$/*Epas1*$^{LSL/+}$). For dimethyloxaloylglycine (DMOG) study, 6- to 8-week-old ODD-luciferase mice (n = 3) were intraperitoneally injected with 400 mg/kg DMOG daily or saline for 2 days, a regimen previously shown to stabilize HIF-1α in vivo.[11] For tumor necrosis factor (TNF)–α inhibition study, 6- to 8-week-old *Vhl*$^{F/F}$ and *Vhl*$^{\Delta IE}$ mice were subcutaneously injected with 5 mg/kg Enbrel (Immunex Corporation, Thousand Oaks, CA) 4 hours before *Citrobacter rodentium* infection, and at day 1 and day 4 after *C rodentium* infection, or intraperitoneally injected with 10 mg/kg Remicade (Janssen Biotech, Inc., Horsham, PA) 1 day and 4 hours before and every other day after *C rodentium* infection or dextran sulfate sodium (DSS) treatment. All mice were maintained in standard cages in a light and temperature-controlled room and were given standard chow and water ad libitum. All animal studies were carried out in accordance with Institute of Laboratory Animal Resources guidelines and approved by the University Committee on the Use and Care of Animals at the University of Michigan (UCUCA approval number: 10299).

Results

EPAS1 Is Activated in Mouse Models of Colitis and Human IBD

In order to test the presence of hypoxia in IBD, the ODD-luciferase hypoxia reporter mouse model was assessed after induction of acute colitis.[14] The ODD-luciferase mice were treated with *C rodentium*, a model of enteric infection–induced colitis, or dextran sulfate sodium (DSS), an injury-induced colitis model.[20,21] In vivo imaging demonstrated that the luciferase signal is increased in the abdomen of *C rodentium* or 3%-DSS–treated mice (Figure 1*A*). This increase was consistent with a robust activation of luciferase signal in excised colons from the ODD-luciferase mice after *C rodentium* or DSS treatment. EPAS1 expression was further assessed in colon samples from both murine experimental colitis and IBD patients by immunohistochemical staining (Figure 1*B*). In untreated mice and normal control human intestines there was a diffuse background staining by the EPAS1 antibody. However, it is clear that in inflamed mouse colon after *C rodentium* or 3% DSS treatment or in UC or CD samples, there was a robust increase in nuclear EPAS1 staining, which colocalizes to an epithelial-specific marker, E-cadherin. Western blot analysis further confirmed an increase in EPAS1 expression in individual UC and CD patients (Figure 1*C*). These results indicate that EPAS1 can play a critical role in the progression of IBD.

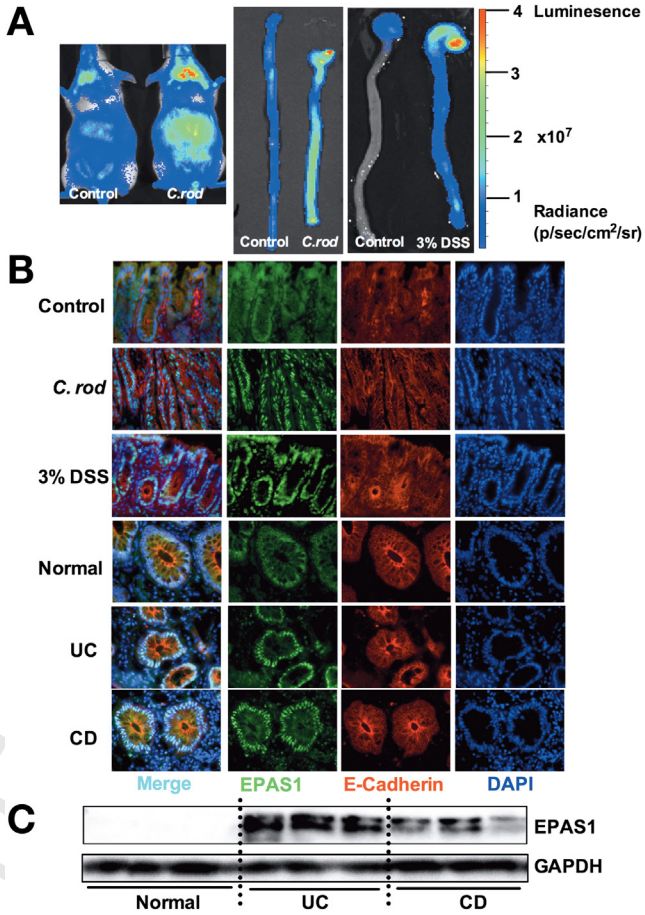

Figure 1. Activation of EPAS1 in mouse models of colitis and IBD. (*A*) Whole animal or excised colon luminescent imaging of ODD-luciferase mice after *C rodentium* infection for 11 days or 3% DSS treatment for 3 days, or vehicle treatment (control). Luminescence intensity is scaled for radiance according to scale bars with gradient color peaks. (*B*) Immunohistochemical staining for the expression of EPAS1 and E-cadherin in colons from mouse models of colitis (*C rodentium* and DSS) or control mice and ulcerative colitis (UC), Crohn's disease (CD), or normal colon tissues. (*C*) Western blotting analysis for the expression of EPAS1 in UC, CD, or normal colon tissues.

Disruption of EPAS1 Protects Mice From DSS-Induced Colonic Damage

Since EPAS1 expression was rapidly activated in epithelial cells in acute models of colitis and predominantly observed in epithelium in UC and CD, mice with an intestinal epithelial-specific deletion of *Epas1* (*Epas1*$^{\Delta IE}$) were assessed after DSS treatment (Figure 2, Supplementary Figure 1). After 7 days of 3% or 5% DSS treatment no difference in body weight was observed between *Epas1*$^{\Delta IE}$ and *Epas1*$^{F/F}$ mice (Supplementary Figure 1*A–C*). Western blot analysis from scraped mucosal samples demonstrated that the expression of EPAS1 in the colon was induced in wild-type littermate mice (*Epas1*$^{F/F}$) with 3% or 5% DSS treatment for 3 days, while this induction was inhibited in *Epas1*$^{\Delta IE}$ mice (Figure 2*A*, Supplementary Figure 1*D*). These data provide additional evidence that an increase of EPAS1 after acute insult is primarily due to EPAS1 activation in intestinal