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Signal Transducer and Activator of Transcription 5b Promotes Mucosal Tolerance in Pediatric Crohn's Disease and Murine Colitis

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Growth hormone (GH) regulates anabolic metabolism via activation of the STAT5b transcription factor and reduces mucosal inflammation in colitis. Peroxisome proliferator-activated receptor (PPAR) γ suppresses mucosal inflammation and is regulated by GH through STAT5b. We hypothesized that the GH:STAT5b axis influences susceptibility to colitis via regulation of local PPARγ abundance. Colon biopsies from children with newly diagnosed Crohn's disease (CD) and controls were exposed to GH in short-term organ culture. Trinitrobenzene sulfonic acid (TNBS) administration was used to induce colitis in STAT5b-deficient mice and wild-type controls, with and without rosiglitazone pretreatment. GH receptor, STAT5b, PPARγ, and nuclear factor κB activation and expression were determined. Epithelial cell GH receptor expression and GH-dependent STAT5b activation and PPARγ expression were reduced in CD colon. STAT5b-deficient mice exhibited reduced basal PPARγ nuclear abundance and developed more severe proximal colitis after TNBS administration. This was associated with a significant increase in mucosal nuclear factor κB activation at baseline and after TNBS administration. Rosiglitazone ameliorated colitis in wild-type mice but not STAT5b-deficient mice. GH-dependent STAT5b activation is impaired in affected CD colon and contributes to chronic mucosal inflammation via down-regulation of local PPARγ expression. Therapeutic activation of the GH:STAT5b axis therefore represents a novel target for restoring both normal anabolic metabolism and mucosal tolerance in CD. ***(Am J Pathol 2006, 169:1999–2013; DOI: 10.2353/ajpath.2006.060186)***

Current evidence suggests that Crohn's disease (CD) is caused by loss of tolerance to the enteric flora, leading to chronic inflammation and intestinal damage.[1] Increased epithelial nuclear factor κB (NF-κB) activity and consequent chemokine expression are fundamental molecular features of this loss of tolerance. Increased NF-κB DNA-binding activity has been attributed to increased binding of the p50 and p65 subunits.[2] Importantly, disease activity in mice with colitis due to 2,4,6-trinitrobenzene sulfonic acid (TNBS) administration was inhibited by antisense oligonucleotides that inhibit p65 expression.[2] These studies suggest a critical role for NF-κB in mediating expression of proinflammatory factors, which are central to the pathogenesis of CD. Although much less is known regarding the regulatory transcription factors that may promote mucosal tolerance, recent studies have suggested that both STAT5a/b and peroxisome proliferator-activated receptor (PPAR) γ may play a role.

Colonic mRNA expression of the STAT5b transcription factor is down-regulated in affected segments in adults with CD and ulcerative colitis.[3] However, the functional significance of this is not known. Moreover, whether GH-dependent STAT5b tyrosine phosphorylation and DNA binding are reduced in affected CD colon has not been determined. Current data suggest that the closely related STAT5a and STAT5b transcription factors are required, in

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a largely redundant manner, for the survival of anti-inflammatory regulatory T cells (Treg) and maintenance of mucosal tolerance. Combined deletion of STAT5a and STAT5b results in reduced survival of Treg and the spontaneous development of autoimmune disease including colitis.[4] Targeted deletion of STAT5b alone results in growth failure due to impaired growth hormone (GH) signaling, without spontaneous autoimmune disease.[5–8] A nonredundant role for STAT5b in preventing mucosal inflammation has not previously been proposed. Although our group and others have shown that GH administration will improve symptoms in patients with CD and reduce mucosal inflammation in several animal models of colitis, whether this involves mucosal STAT5b activation is not known.[9–14]

PPARγ is a nuclear receptor that is highly expressed in colon epithelial cells (CECs) and lamina propria macrophages (LPMs) and that promotes mucosal tolerance by inhibiting NF-κB activation.[15,16] Recently, PPARγ has been shown to inhibit NF-κB activity by associating with the p65 subunit in the nucleus and promoting export of the PPARγ:p65 complex to the cytosol.[16] Multiple experimental studies using PPARγ haplo-insufficient mice and PPARγ gene therapy have confirmed a profound anti-inflammatory effect in murine colitis.[15,17–19] Patient-based studies have demonstrated a reduction in PPARγ expression in inflamed colon, with mixed results obtained with PPARγ ligand administration.[20–23] Administration of the PPARγ agonist rosiglitazone has also been more consistently effective when given before the onset of experimental colitis than when given after the onset of disease.[15,18,19,24] This may be due to the basal reduction in PPARγ nuclear abundance, preventing an optimal response to ligand administration. The molecular basis for the reduction in PPARγ expression in colitis has not been defined. Recently, GH has been shown to regulate the human PPARγ3 transcript, which is highly expressed in CECs and LPMs, via a STAT5b *cis*-element.[25] Interestingly, STAT5a could not substitute for STAT5b in this regard. Whether STAT5b, via regulation of PPARγ nuclear abundance, would exert a nonredundant tolerogenic effect in colitis was not known.

In this study, we have determined that STAT5b activation and PPARγ expression are reduced in pediatric CD colon at diagnosis. STAT5b-deficient mice exhibited reduced colonic PPARγ nuclear abundance, increased NF-κB activation, and enhanced susceptibility to colitis after TNBS administration; this was refractory to rosiglitazone administration.

Materials and Methods

Human GH was from Sigma (St. Louis, MO). All antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA) unless otherwise noted. Anti-GH receptor $(GHR)_{cyt\text{-}AL47}$ is a polyclonal antibody against the human GHR cytoplasmic domain that cross reacts with mouse GHR.[26] This serum and its control preimmune serum were purified by ammonium sulfate precipitation. Antibodies specific for STAT5a and STAT5b were from Zymed Laboratories (South San Francisco, CA). A tyrosine phosphorylation-specific STAT5 antibody was from Upstate Biotechnology (Lake Placid, NY). Rosiglitazone was from Cayman Chemicals (Ann Arbor, MI) and was blended with the chow food at 80 mg/kg food to provide 20 mg/kg/day to a 20-g mouse consuming 5 g of chow food per day. We confirmed that wild-type (WT) and STAT5b-deficient mice consumed sufficient chow in the 3 days before TNBS administration to receive 20 mg/kg rosiglitazone per day.

Patient-Based Studies

Colon biopsies were obtained from untreated pediatric patients with Crohn's colitis and healthy controls 5 to 18 years of age during the initial diagnostic colonoscopy. Biopsies were obtained from endoscopically affected segments primarily in the cecum and ascending colon. The diagnosis of CD was made by the patient's primary gastroenterologist based on established clinical, radiological, and histological criteria. The study was approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board and the Cincinnati Children's Hospital Medical Center General Clinical Research Center Scientific Advisory Committee. The Crohn's Disease Histological Index of Severity (CDHIS) was computed as previously described by a single gastrointestinal pathologist (E.B.).[27,28]

Human Colon Biopsy Organ Culture

In brief, colon biopsies were washed with cold phosphate-buffered saline (PBS) and trimmed into explants.[10] Explants were cultured in serum-free CMRL-1066 tissue culture medium (GIBCO, Grandland, NY) on collagen I-coated plates (BIOCOAT, Bedford, MA) at 37°C in 95% oxygen, 5% carbon dioxide atmosphere for 24 hours. The morphological appearance of the explants was preserved under these conditions as assessed by light microscopy. After 24 hours in culture, explants were treated with human GH (500 ng/ml) or PBS, and nuclear protein and sections were prepared 45 minutes later. This dose of human GH has previously been shown to regulate chloride secretion in the T84 human colon carcinoma cell line and to activate STAT5 in liver cell lines.[29,30]

Animal Resources and Maintenance

STAT5b-deficient mice were provided by Dr. James Ihle (St. Jude Children's Research Hospital, Memphis, TN).[8] These mice are on a C57BL6/J background; sex-matched males and females were used between 8 and 12 weeks of age. The protocol was approved by the Cincinnati Children's Hospital Research Foundation Institutional Animal Care and Use Committee.

TNBS Colitis

TNBS colitis was induced as previously described.[31,32] One or 2 mg of TNBS in 50% ethanol was administered in