Growth Hormone Inhibits Signal Transducer and Activator of Transcription 3 Activation and Reduces Disease Activity in Murine Colitis

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Background & Aims: **Constitutive signal transducer and activator of transcription (STAT) 3 activation promotes chronic inflammation and epithelial proliferation in murine colitis and human inflammatory bowel disease. SHP-2, through binding to the glycoprotein 130 signaling receptor, negatively regulates STAT3 activation. Growth hormone reduces disease activity and promotes mucosal healing in colitis and can activate SHP-2.** ***Methods:*** **We hypothesized that growth hormone administration would reduce disease activity in experimental colitis and that this would involve modulation of SHP-2/glycoprotein 130 association and STAT3 activation.** ***Results:*** **Growth hormone administration improved weight gain and colon histology in interleukin 10–null mice with colitis. Growth hormone reduced apoptosis and increased proliferation of crypt epithelial cells while increasing apoptosis of lamina propria mononuclear cells. Growth hormone increased SHP-2/glycoprotein 130 association and reduced colonic STAT3 activation in interleukin 10–null mice and in biopsy samples from patients with Crohn's colitis. Expression of the antiapoptotic protein bcl-2 was increased in crypt epithelial cells after growth hormone treatment. Growth hormone increased SHP-2/glycoprotein 130 binding and reduced interleukin 6–dependent STAT3 activation in the T84 human colon carcinoma and Jurkat human T-cell leukemia lines.** ***Conclusions:*** **Growth hormone administration improves weight gain and reduces disease activity in interleukin 10–null mice with colitis. The improvement in disease activity is associated with increased SHP-2/glycoprotein 130 binding and reduced STAT3 activation in both murine and Crohn's colitis. Growth hormone may be a useful therapy in inflammatory bowel disease, in terms of both improving anabolic metabolism and enhancing mucosal healing.**

Growth hormone (GH) administration alleviates symptoms in patients with Crohn's disease (CD), although effects on mucosal healing have not been determined.[1] Experimental studies have shown reduced mucosal inflammation and accelerated epithelial healing with GH treatment in colitis due to dextran sodium sulfate (DSS) or trinitrobenzene sulfonic acid (TNBS) administration.[2,3] The underlying mechanism has not been established, although a role for increased circulating insulin-like growth factor (IGF)-I has been proposed.[3] However, the GH/IGF-I axis has also been implicated in the development of colorectal carcinoma; this has also been associated with increased circulating IGF-I.[4] The risk of colorectal cancer is increased in long-standing inflammatory bowel disease (IBD), particularly in severe ulcerative colitis.[5] Therefore, a better understanding of the molecular basis for the effects of GH on inflammation and epithelial repair in colitis will be critically important in terms of potentially translating this therapy to patients with IBD.

Upon binding to its cell-surface receptor, GH induces autophosphorylation of Janus kinase 2, which, in association with the GH receptor (GHR), activates via tyrosine phosphorylation the signal transducer and activator of transcription (STAT)5b transcription factor.[6] Activated STAT5b translocates to the nucleus and up-regulates anabolic target genes, including IGF-I.[7] Most circulating IGF-I is synthesized in the liver, although a low level of GH-dependent and -independent IGF-I expression has been shown in extrahepatic tissues, including colon.[8,9] Anabolic effects of GH, including increases in feed efficiency (weight gain normalized to caloric intake), are primarily mediated via IGF-I.[10] Recent data from a

Abbreviations used in this paper: CEC, crypt epithelial cell; DSS, dextran sodium sulfate; EMSA, electrophoretic mobility shift assay; FasL, Fas ligand; GH, growth hormone; GHR, growth hormone receptor; gp, glycoprotein; IGF, insulin-like growth factor; IL, interleukin; LPMC, lamina propria mononuclear cell; PCNA, proliferating cell nuclear antigen; PCR, polymerase chain reaction; SHP, Src homology-2 domain–containing phosphatase; SH-PTP, Src homology-2 domain–containing protein tyrosine phosphatase; sIL-6R, soluble IL-6 receptor; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription; TNBS, trinitrobenzene sulfonic acid; TUNEL, terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling; WT, wild type.

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liver-specific IGF-I–null mouse have shown that, whereas circulating IGF-I contributes to linear growth and bone formation, extrahepatic IGF-I production can support virtually normal postnatal growth.[9,11] Whether the beneficial effects of GH on either anabolic metabolism or mucosal healing in colitis are mediated by circulating or local IGF-I is not known.

Colon crypt epithelial cell (CEC) injury by activated lamina propria mononuclear cells (LPMCs) is central to the pathogenesis of IBD.[12] The GHR is expressed on colon CECs and LPMCs, so GH may exert direct effects on mucosal inflammation and healing in colitis.[13,14] Recently, GH has been shown to stimulate proliferation, modulate chloride secretion, and reduce apoptosis in human colon epithelial cell lines.[15,16] In vitro studies have shown both stimulatory and inhibitory effects of GH on monocyte and lymphocyte function.[17] Peripheral blood monocytes from patients with GH deficiency have an activated phenotype, with increased production of tumor necrosis factor α and interleukin (IL)-6; monocyte activation is partially inhibited by GH-replacement therapy.[18] Although GH has consistently been shown to reduce inflammation and accelerate mucosal healing in experimental colitis, specific molecular effects on LPMCs or CECs have not been characterized.

STAT3 is constitutively activated in the colon in IBD and in murine colitis due to IL-10 deficiency or TNBS administration.[19] This renders activated lamina propria T cells resistant to apoptosis and contributes to ongoing CEC proliferation.[19,20] Dysregulated STAT3 activation has been linked to the development of T-cell lymphoma and epithelial carcinoma.[21,22] Blockade of IL-6 trans-signaling reduces STAT3 activation, induces apoptosis of activated LPMCs, and ameliorates colitis due to IL-10 deficiency.[19] Blockade of IL-6 signaling and STAT3 activation has recently also been shown to inhibit colorectal tumor formation in DSS colitis.[23] IL-6 activates STAT3 via the glycoprotein (gp)130 signaling receptor. Src homology-2 domain–containing phosphatase (SHP)-2 and suppressor of cytokine signaling (SOCS)-3 are negative regulators of gp130-dependent STAT3 activation and use the same docking site on gp130: pY757.[24–26] Studies in extraintestinal tissues have shown activation of SHP-2 and up-regulation of SOCS-3 by GH.[27] Whether GH reduced inflammation in colitis via modulation of SHP-2 or SOCS-3 activity and associated STAT3 activation in LPMCs is not known.

CECs undergo apoptosis in colitis in part via the Fas/Fas ligand (FasL) pathway.[12,28] Fas, the cell-surface receptor for FasL, is constitutively expressed on CECs; expression increases in colitis. FasL is up-regulated on activated T cells and CECs in ulcerative colitis and several experimental models of colitis and induces apoptosis of CECs.[12,28] Targeted disruption of Fas protects CECs from apoptosis in colitis due to IL-2 deficiency, despite ongoing mucosal inflammation.[28] In most tissues examined, GH has been shown to exert an antiapoptotic, proproliferative effect that has both IGF-I–dependent and –independent components.[16] This has included inhibition of Fas-dependent apoptosis via up-regulation of the antiapoptotic protein bcl-2 and down-regulation of the proapoptotic protein bax.[16,29] Trophic effects of GH on small-bowel mucosa are associated with an increase in cell survival, whereas effects of IGF-I include increased cell proliferation.[30,31] Recently, GH has been shown to protect normal colon epithelium from radiation- or chemotherapy-induced injury by reducing cellular apoptosis and increasing proliferation.[32] The effect of GH on CEC apoptosis and bcl-2/bax expression in colitis had not previously been reported.

We hypothesized that GH would reduce mucosal inflammation and promote healing in experimental colitis by down-regulating constitutive STAT3 activation and that this would involve up-regulation of SHP-2/gp130 binding. We have determined that GH promotes anabolic metabolism and reduces disease activity in colitis and that this is associated with reduced STAT3 activation in lamina propria T cells and CECs.

Methods

Materials

T84 human colon carcinoma cells, Jurkat human T-cell leukemia cells (clone E6-1), and IM-9 B-lymphoblast cells were obtained from the American Type Culture Collection (Rockville, MD). Rat GH was obtained from Dr Alfred Parlow, National Hormone & Peptide Program (Harbor-University of California–Los Angeles Medical Center, Torrance, CA). Human GH was obtained from Sigma (St Louis, MO). Human IL-6, soluble IL receptor (sIL-6R), and IL-2 were obtained from R&D Systems (Minneapolis, MN). STAT5b, STAT3, gp130, actin, proliferating cell nuclear antigen (PCNA), SOCS-3, PY20, Src homology-2 domain–containing protein tyrosine phosphatase (SH-PTP) 1, SH-PTP2, bax, bcl-2, fas, fasL, ki-67, and anti-goat tyrosine phosphorylation state-specific STAT3 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and CD3 was obtained from abCam (Cambridge, UK). The GHR AL-47 polyclonal antibody that recognizes the cytoplasmic domain of the GHR was from Dr Stuart Frank (University of Alabama at Birmingham). Anti-rabbit tyrosine phosphorylation state-specific STAT3 and STAT5b antibodies were from Upstate Biotechnology (Lake Placid, NY), and cleaved caspase 3 was from Cell Signaling (Beverly, MA). Secondary antibodies were from Santa Cruz Biotechnology and Jackson ImmunoResearch Laboratories (West Grove, PA). Primers and reagents for real-time poly-