

ABSTRACT

Background: Interleukin-6 (IL-6) activation of the STAT3 transcription factor contributes to chronic inflammation in murine colitis and human Inflammatory Bowel Disease (IBD); Suppressors of Cytokine Signaling 3 (SOCS-3) and SH-PTP2 are negative regulators of this pathway. Growth hormone (GH) has been shown to reduce disease activity and promote mucosal healing in colitis. Anabolic effects of GH are mediated via activation of STAT5; GH target genes include Insulin-Like Growth Factor 1 (IGF-1) and SOCS-3, while SH-PTP2 is activated by GH and can modulate IL-6 signaling by binding to the gp130 IL-6 co-receptor. However, the molecular basis for a beneficial effect of GH in colitis was not known. We **hypothesized** that GH would ameliorate disease activity in colitis, and that this would involve modulation of STAT3/5 activation. **Methods:** Interleukin-10 null (IL-10) mice with colitis and wild type (WT) controls received single (1 mcg/gm IP 30 minutes prior to sacrifice) or chronic (3 mcg/gm SQ BID for two weeks) dose GH administration or PBS. Weight gain, colon histopathology, and STAT3/5, SOCS-3, SH-PTP2, and IGF-1 activation and/or expression were determined. T84 human colon carcinoma cells were treated with IL-6 ± GH and STAT3 & SH-PTP2:gp130 activation were determined. **Results:** GH administration improved weight gain and colon histopathology in colitic mice. The improvement in colon histopathology was significantly associated with a reduction in STAT3 activation. GH administration activated STAT5 and up regulated IGF-1 in colon of WT mice; STAT5 and IGF-1 were constitutively induced in colitic mice, without additional up regulation by GH. STAT3 and SOCS-3 were also constitutively up regulated in colitis, in both colon epithelial cells (CEC) and lamina propria mononuclear cells (LPMC); GH administration significantly reduced STAT3 activation and SOCS-3 abundance in both cell populations; this was associated with induced binding of SH-PTP2 to the IL-6 co-receptor gp130. GH treatment also induced SH-PTP2:gp130 binding and reduced IL-6 dependent STAT3 activation in T84 cells. **Conclusions:** GH administration improved weight gain and reduced disease activity in IL-10 null mice with colitis. The improvement in disease activity was related to a reduction in colonic STAT3 activation, which was associated with increased SH-PTP2:gp130 binding. GH may be a useful adjunct therapy in IBD, both in terms of improving growth and body composition and in enhancing mucosal healing.

BACKGROUND

- **IL-6 dependent STAT3 activation contributes to T cell activation and epithelial cell proliferation in colitis**
- **SOCS-3 and SH-PTP2 negatively regulate IL-6 dependent STAT3 activation**
- **Growth hormone (GH) can enhance mucosal healing in colitis**
- **Anabolic effects of GH are mediated via STAT5 activation & IGF-1 expression**
- **GH can also up regulate SH-PTP2 and SOCS-3**

HYPOTHESIS

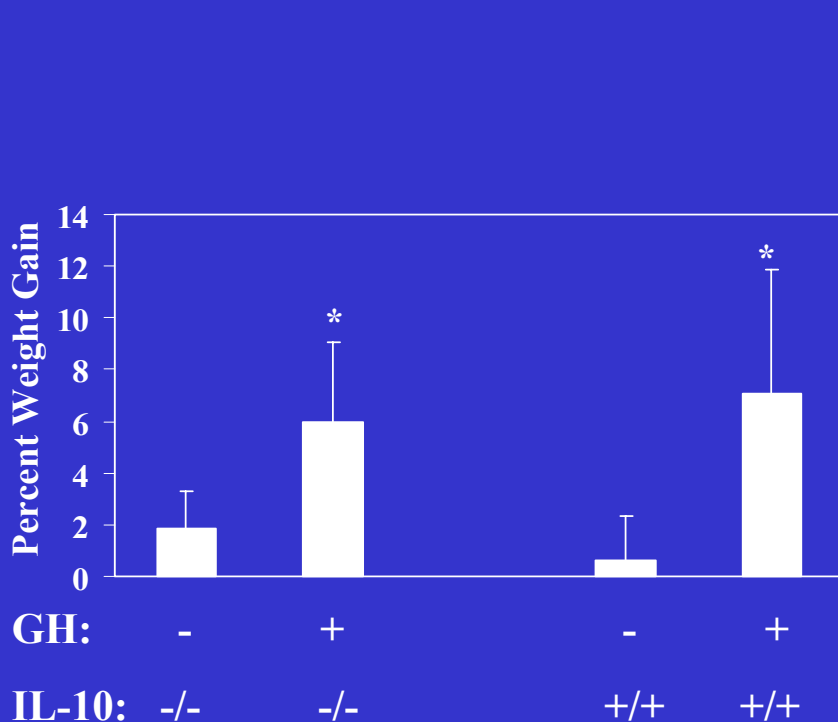
GH ameliorates disease activity in colitis via modulation of STAT3 activation

METHODS

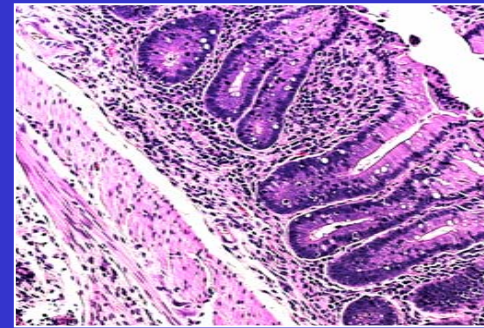
- Wild type and IL-10 null C3H/HeJBir mice received PBS or single (1 mcg/gm IP) or chronic (3 mcg/gm SC for two weeks) dose GH administration.
- Weight gain, body composition, and colon histopathology were determined.
- Colon & liver STAT3/5, gp130, GHR, SH-PTP2, SOCS-3, and IGF-1 activation, expression, and/or localization were determined by real time PCR, immunoblot, and immunohistochemistry.
- T84 human colon carcinoma cells were treated with IL-6 ± GH and STAT3 activation and SH-PTP2:gp130 binding were determined.

RESULTS

GH Improves Weight Gain & Reduces Disease Activity in Colitis



PBS score: 8.8 ± 2.5



GH score: $6.1 \pm 3^*$

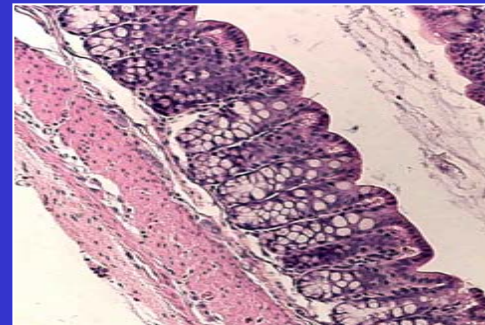


Figure 1. PBS or GH were administered for two weeks to wild type and IL-10 null mice & weight gain and colon histopathology score were determined. * $p < .05$ vs PBS control

GH does not increase liver IGF-1 expression in colitic mice

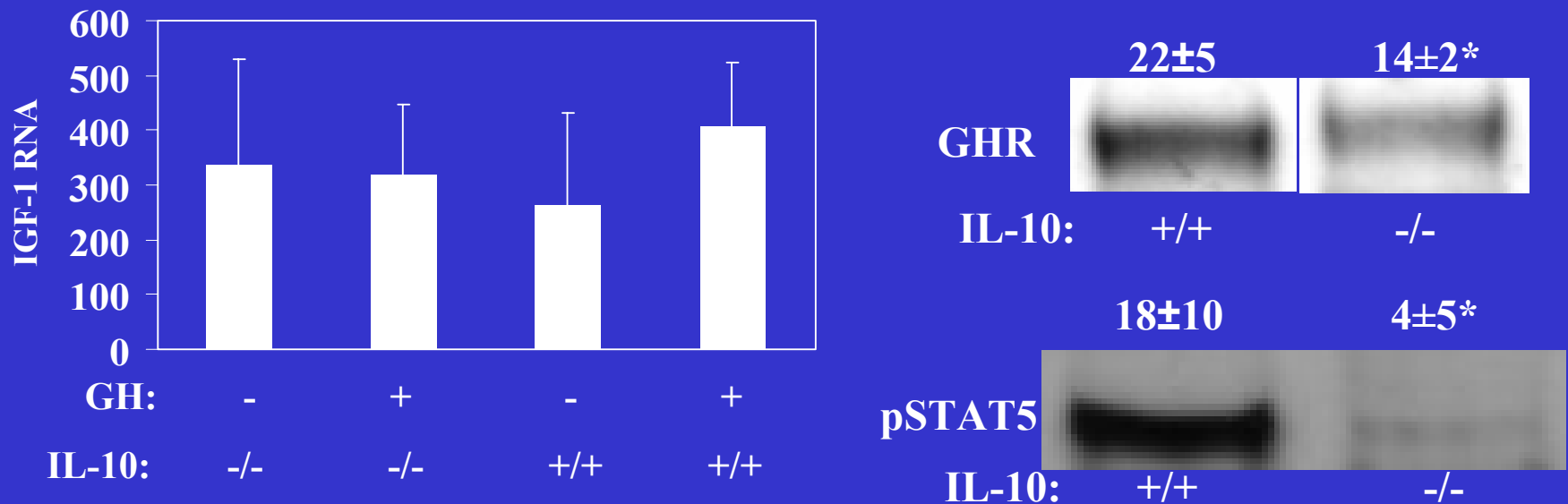


Figure 2. Liver IGF-1 expression and GHR and phospho-STAT5 abundance were determined in wild type and IL-10 null mice \pm GH administration. Mean \pm SD for signal intensity are shown for GHR & pSTAT5, n=4-8, *p<.05 versus control.

GH does not activate colon STAT5 or IGF-1 in colitic mice

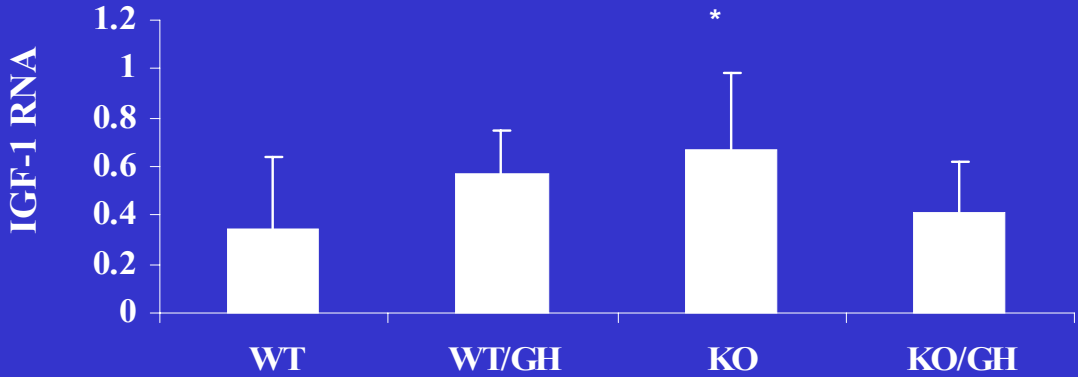
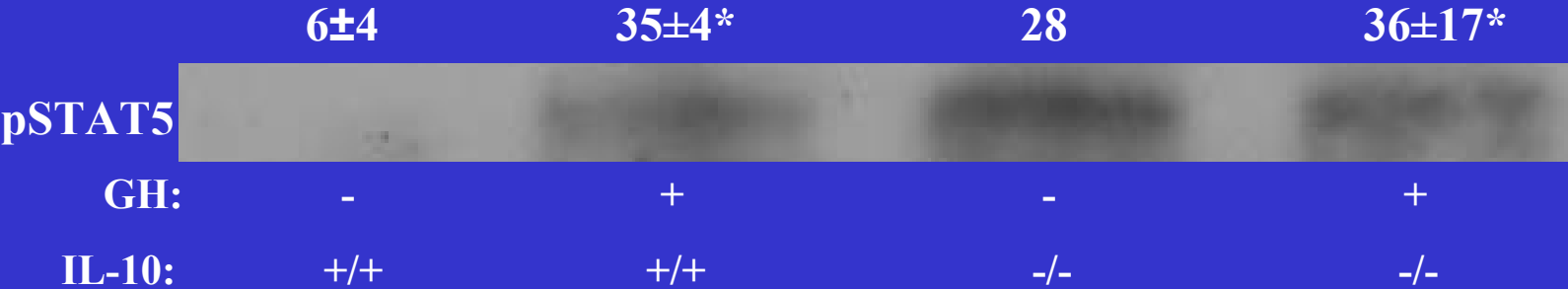


Figure 3. Colon phospho-STAT5 abundance and IGF-1 expression were determined in wild type and IL-10 null mice ± GH administration. Mean ±SD for signal intensity are shown for pSTAT5, n=4-8, *p<.05 versus IL-10 +/+ control.

GH reduces colon STAT3 activation

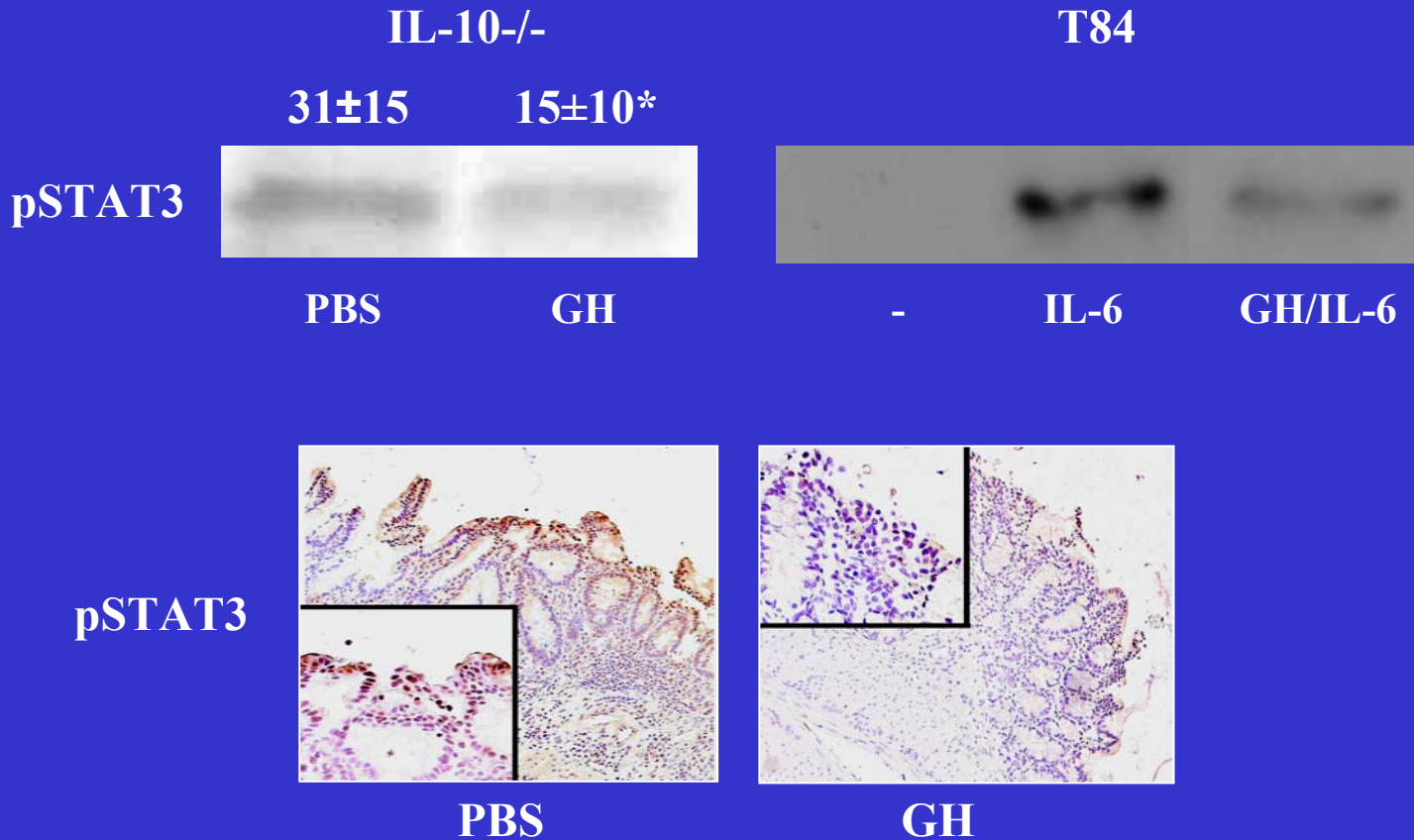


Figure 4. Colon and T84 cell phospho-STAT3 abundance/localization were determined in IL-10 null mice \pm GH administration or T84 cells treated with IL-6 \pm GH. Mean \pm SD for signal intensity are shown for pSTAT3, n=4-8, *p<.05 versus control.

Reduced colon STAT3 activation is associated with improved histopathology

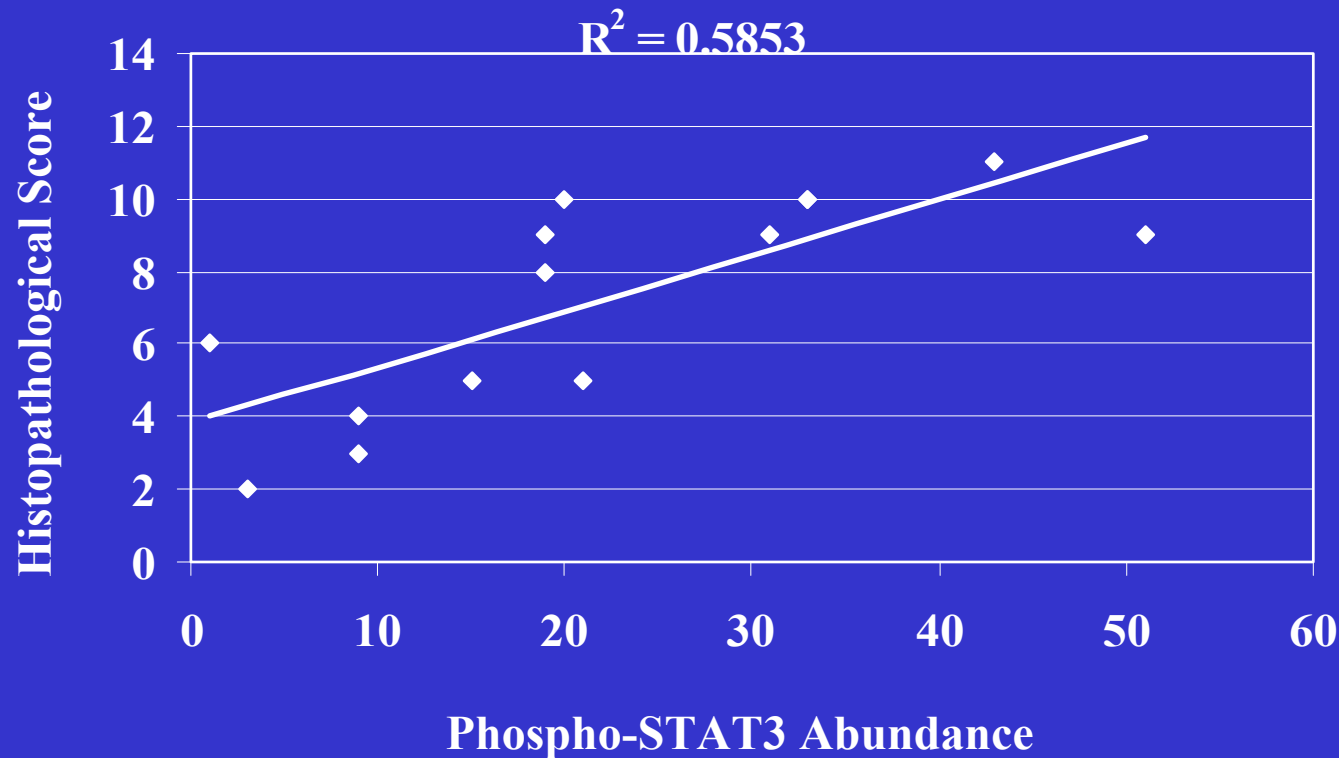


Figure 5. Colon phospho-STAT3 abundance and histopathology scores were determined in IL-10 null mice treated with PBS or GH for two weeks.

GH reduces colon SOCS-3 expression & abundance

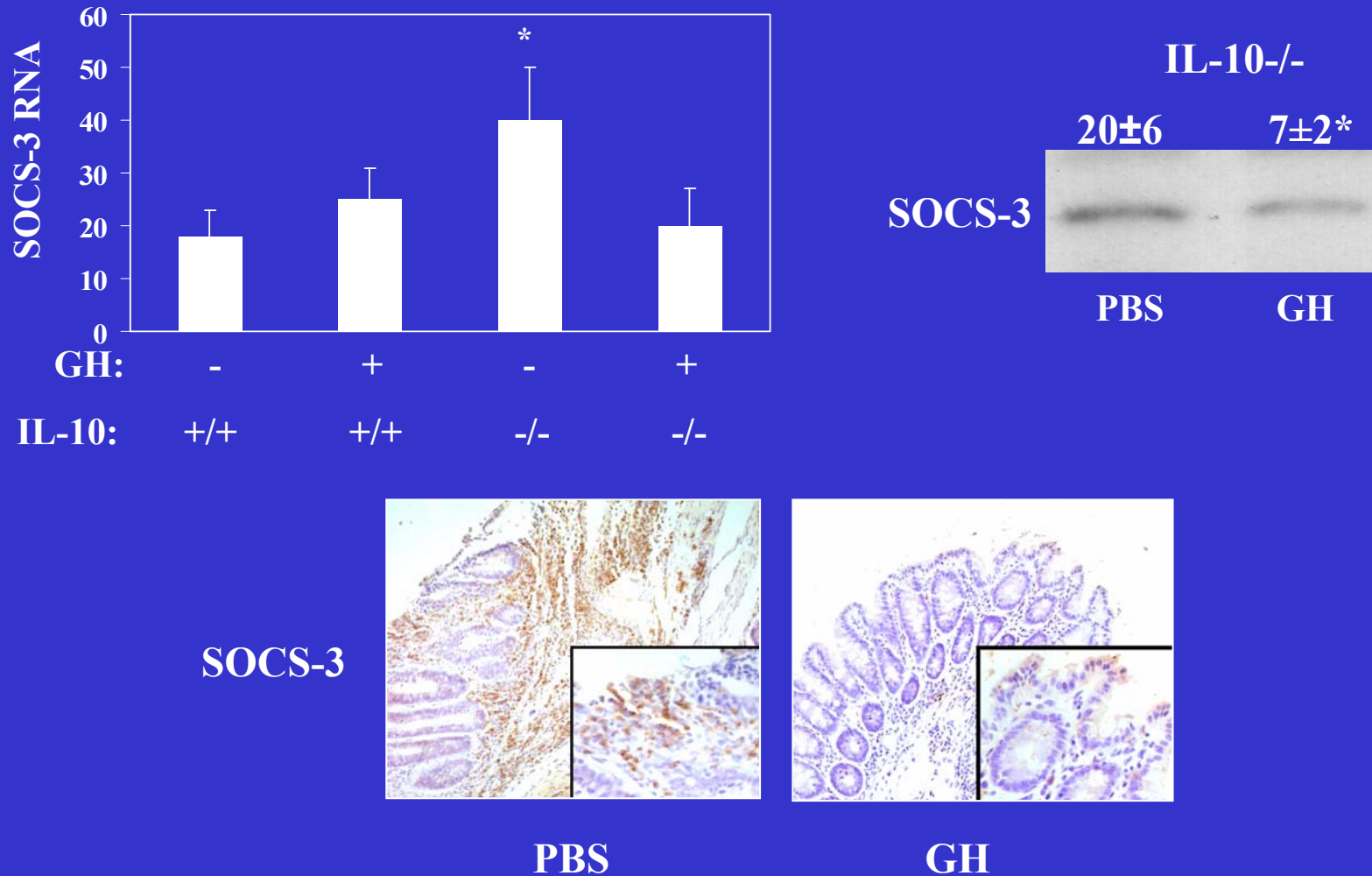


Figure 6. Colon SOCS-3 RNA expression and protein abundance/localization were determined in IL-10 null mice \pm GH administration Mean \pm SD for signal intensity is shown for SOCS-3 protein, n=4-8, *p<.05 versus control.

GH induces SH-PTP2 association with gp130 & inhibits IL-6 activation of STAT3

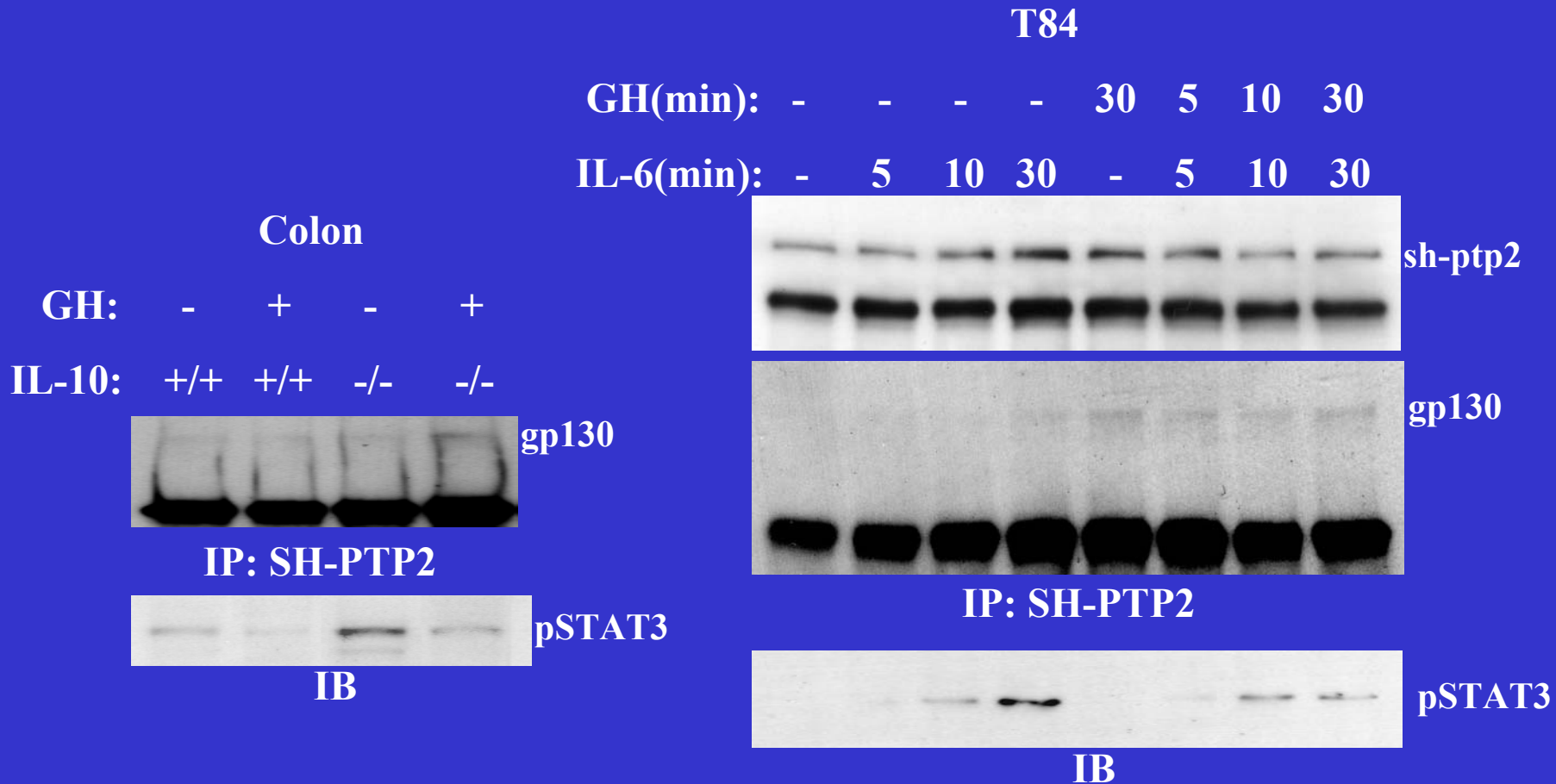


Figure 7. GH dependent SH-PTP2 association with the IL-6 signaling co-receptor gp130 & IL-6 dependent STAT3 phosphorylation were determined in mouse colon and/or T84 cells. IP: antibody used for immunoprecipitation; IB:pSTAT3 immunoblot.

CONCLUSIONS

- **GH administration improves weight gain & reduces disease activity in experimental colitis**
- **GH reduces colon STAT3 activation**
- **Reduced STAT3 activation is associated with GH induction of SH-PTP2:gp130 binding**
- **Future studies will examine potential anabolic & immunomodulatory effects of GH in Crohns Disease**

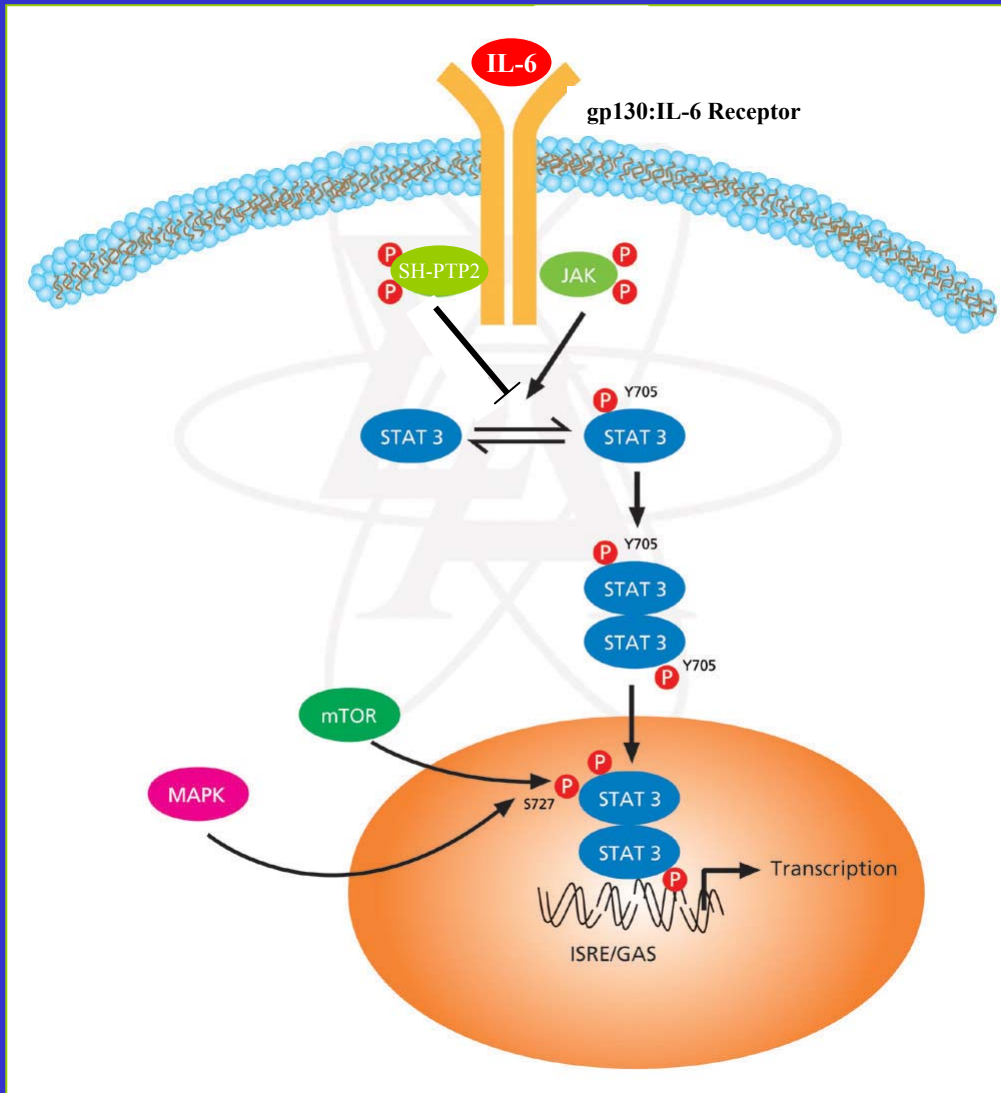


Figure 8. GH administration leads to activation of SH-PTP2 and increased SH-PTP2:gp130 binding. This may then reduce IL-6 dependent STAT3 activation and associated inflammation & crypt hyperplasia in colitis.