INFLAMMATION

Tumour necrosis factor α blockade induces an anti-inflammatory growth hormone signalling pathway in experimental colitis

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Background: Neutralisation of tumour necrosis factor α (TNF α) restores systemic growth hormone function in patients with Crohn's disease, and induces mucosal healing. Anabolic effects of growth hormone depend on activation of the STAT5 transcription factor. Although it has recently been reported that both administration of growth hormone and neutralisation of TNF α reduce mucosal inflammation in experimental colitis, whether this involved activation of STAT5 in the gut is not known.

Aim: To determine whether TNF α blockade in colitis up regulates a growth hormone:STAT5 signalling pathway in the colon.

Methods: Interleukin 10-deficient mice and wild-type controls received growth hormone or anti-TNF α antibody, and T84 human colon carcinoma cells were treated with TNF α or growth hormone. Activation and expression of STAT5b, peroxisome proliferator-activated receptor gamma (PPAR γ), NF κ B/I κ B and growth hormone receptor were determined.

Results: Growth hormone activated STAT5b and up regulated expression of PPAR γ in normal mouse colon; inflamed colon was partially resistant to this. Chronic administration of growth hormone, nevertheless, significantly reduced activation of colonic NF κ B ($p=0.028$). Neutralisation of TNF α rapidly increased abundance of growth hormone receptor, activation of STAT5 and abundance of PPAR γ in the colon, but reduced activation of NF κ B in colitis. Growth hormone activated STAT5, and directly reduced TNF α activation of NF κ B, in T84 cells.

Conclusions: Reduced activation of colonic STAT5 and expression of PPAR γ may contribute to persistent mucosal inflammation in colitis. Up regulation of STAT5 and PPAR γ , either through neutralisation of TNF α or chronic administration of growth hormone, may exert an anti-inflammatory effect in inflammatory bowel disease.

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Patients with Crohn's disease exhibit hepatic resistance to the action of growth hormone, manifested by low circulating levels of insulin-like growth factor 1 (IGF1) despite adequate central growth hormone secretion.¹ This contributes to cachexia, reduced bone mineral density and linear growth failure. Administration of the monoclonal anti-tumour necrosis factor α (TNF α) antibody, infliximab, restores normal circulating levels of IGF1 within 24 h, consistent with restoration of growth hormone signalling in the liver.^{2–3} This leads to improvements in growth and bone mineral density over time.⁴ Our group recently reported that expression of growth hormone receptor (GHR) and activation of growth hormone-dependent STAT5b in the liver are reduced in interleukin 10 (IL10)-deficient mice with colitis, providing a molecular basis for the hepatic resistance to growth hormone that is observed in patients. TNF α neutralisation up regulated abundance of GHR in the liver and activation of growth hormone-dependent STAT5b and serum IGF1 within 24 h, mimicking the results observed in patients with Crohn's disease.⁵ However, whether activation of STAT5 in the affected colon would similarly increase after TNF α blockade in colitis is not known.

Administration of growth hormone reduces mucosal inflammation in several animal models of colitis, and alleviates symptoms in patients with Crohn's disease.^{6–9} The GHR is expressed on epithelial and lamina propria cells of the small bowel and colon.¹⁰ However, regulation of growth hormone signalling in the normal or inflamed gut is not well understood. Although there are several intracellular targets for growth hormone signalling in the liver, activation of STAT5b has been shown to regulate expression of IGF1 and linear growth in a

non-redundant manner.¹¹ Recently, growth hormone has also been shown to up regulate the peroxisome proliferator-activated receptor gamma (PPAR γ) 3 isoform in humans, in a manner that involves STAT5b but not STAT5a.¹² The PPAR γ 3 isoform is expressed in colon epithelial cells (CECs) and macrophages, and would be expected to exert a tolerogenic effect in colitis through local inhibition of activation of nuclear factor κ B (NF κ B).^{13–18} Conversely, targeted deletion of both STAT5a and STAT5b is required to produce spontaneous autoimmune disease, including colitis in mice, through decreased survival of regulatory T cells.¹⁹ As growth hormone activates both STAT5a and STAT5b, this raises the possibility that growth hormone signalling might exert a tolerogenic effect in the gut under normal conditions, and that a TNF α -dependent reduction in growth hormone function might promote chronic mucosal inflammation.

The monoclonal anti-TNF α antibody infliximab can considerably reduce mucosal inflammation in Crohn's disease; this has been attributed to apoptosis of lamina propria effector T cells.^{20–25} However, neutralisation of TNF α may also restore an endogenous tolerogenic pathway in the gut. We hypothesised that neutralisation of TNF α would rapidly restore a novel growth hormone-dependent anti-inflammatory pathway in the

Abbreviations: CEC, colon epithelial cells; EMSA, electrophoretic mobility shift assay; GHR, growth hormone receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IGF1, insulin-like growth factor 1; IHC, immunohistochemistry; LPMC, lamina propria mononuclear cells; NF κ B, nuclear factor κ B; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PPAR γ , peroxisome proliferator-activated receptor gamma; TNF, tumour necrosis factor

colon, comprised of activation of STAT5b and nuclear expression of PPAR γ , thereby contributing to mucosal healing. In this study, we determined that neutralisation of TNF α rapidly increases activation of STAT5 and nuclear abundance of PPAR γ in the colon of mice with chronic colitis owing to IL10 deficiency, leading to reduced activation of NF κ B.

MATERIALS AND METHODS

Materials

Human growth hormone was from Sigma (St Louis, Missouri, USA). All antibodies were from Santa Cruz Biotechnology (Santa Cruz, California, USA), unless otherwise noted. Anti-GHR_{CYT-AL47} is a polyclonal antibody to the human GHR cytoplasmic domain that cross reacts with mouse GHR.^{26,27} This serum and its control preimmune serum were purified by ammonium sulphate precipitation. Antibodies specific to STAT5a and STAT5b were from Zymed Laboratories (South San Francisco, California, USA). A tyrosine phosphorylation-specific STAT5 antibody was from Upstate Biotechnology (Lake Placid, New York, USA). Rat/mouse monoclonal anti-TNF α antibody (clone cV1q) and isotype control immunoglobulin G (IgG) antibody (cVaM) were provided by Centocor (Malvern, Pennsylvania, USA).⁵

Animal resources and maintenance

Breeding pairs for C3H/HeJBir IL10^{+/+} (wild type) and C3H/HeJBir IL10^{-/-} mice were provided by Dr Edward Leiter, Jackson Labs (Bar Harbor, Maine, USA).²⁸ The protocol was

approved by the Children's Hospital Research Foundation, Institutional Animal Care & Use Committee.

Administration of growth hormone and anti-TNF α antibody

Administration of growth hormone consisted of a single intraperitoneal injection (1 μ g/g) given 30 min before killing, or a subcutaneous injection (3 μ g/g twice daily) for 2 weeks. Control mice were injected with an equal volume of sterile phosphate-buffered saline (PBS). IL10-null mice received anti-TNF α antibody, 1 mg intraperitoneal, or isotype control IgG antibody, 1 mg intraperitoneal, as a single dose and killed 24 h later.⁵

Cell culture

T84 cells were maintained at 37°C in Dulbecco modified Eagle medium with 10% fetal bovine serum. Subconfluent cells were serum-starved overnight and stimulated with TNF α (100 ng/ml) for 12 h, followed by human growth hormone 500 ng/ml, 30 min, in serum-free media.

RNA isolation and real-time polymerase chain reaction

Total RNA was isolated with Trizol from the whole caecum and proximal colon. The concentration of the total RNA was measured at 260 nm, and quality was checked by determining the 260 nm:280 nm ratio of 1.6:2. A 5 μ g aliquot of RNA was used to perform reverse transcription with the ProSTAR RT-PCR Kit (Stratagene, La Jolla, California, USA); cDNA was used to

Figure 1 Growth hormone (GH) activates STAT5b and up regulates expression of PPAR γ . Interleukin 10 (IL10)-deficient mice and wild-type controls were treated with growth hormone (3 μ g/g twice daily) for 2 weeks, and nuclear proteins, RNA and tissue sections of the colon were prepared. (A) Western blot was performed for nuclear pSTAT5 and STAT5b, and SHP1 as a control for protein loading. Signal intensity was determined by densitometry and is shown as mean (standard deviation (SD)), $n=4$. (B) Immunohistochemistry for pSTAT5 was performed; results with an isotype control antibody are shown in the inset. Representative pSTAT5-positive cells are indicated by the arrows and circle. Original magnification $\times 400$, bar = 50 μ m. (C) Real-time polymerase chain reaction was performed for expression of total PPAR γ mRNA. Results are shown as mean (SD), $n=4$.

Figure 2 Chronic administration of growth hormone (GH) reduces nuclear abundance of p65 in the colon of mice with colitis due to interleukin 10 (IL10) deficiency. (A) Mice with established colitis due to IL10 deficiency and wild-type controls were treated with growth hormone (3 μ g/g twice daily for 2 weeks), and nuclear proteins of the colon were prepared. Abundance of p65 and SHP1 was determined by western blot. (B) Wild-type and IL10-null mice received rat growth hormone (1 μ g/g intraperitoneal, single dose) or PBS 30 min before killing, and nuclear abundance of p65 and SHP1 was determined by western blot. Signal intensity was determined by densitometry and is shown as mean (SD), $n = 4$.

perform SYBER green real-time polymerase chain reaction (PCR) on the Mx4000 multiplex quantitative PCR instrument (Stratagene) following the Brilliant SYBER green QPCR Master Mix manual (Stratagene). The primers used for the SYBER green real-time-PCR assay were PPAR γ : sense: CAG GCT TGC TGA ACG TGA AG, anti-sense: GGA GCA CCT TGG CGA ACA; GAPDH: sense: TTT GGC TAC AGC AAC AGG GTG, anti-sense: GGG TCT CTC TCT TCC TCT TGT GC.^{17–29} GAPDH was amplified to standardise the quantification of target cDNA.

Immunoblot and electrophoretic mobility shift assay

As previously described,⁷ the entire caecum and proximal colon from wild-type and IL10-null mice were homogenised on ice using a Polytron tissue homogeniser; protein was prepared using the NE-PER Kit (Pierce, Rockford, Illinois, USA), and immunoblots were prepared. About 10 μ g of nuclear protein was used to perform the STAT5 (electrophoretic mobility shift assay, EMSA). STAT5 nuclear binding was performed using a duplex oligonucleotide probe based on growth hormone-induced STAT5b DNA-binding element in the IGF1 gene promoter. The oligonucleotide sequences were STAT5 sense: GGG CCT TCC TGG AAG AAA, anti-sense: TTT CTT CCA GGA

AGG CCC. DNA strands were labelled by biotin (Pierce) and STAT5 was detected using a LightShift Chemiluminescent EMSA Kit (Pierce). For supershift, 10 μ g STAT5a/b antibody (Santa Cruz Biotechnology) was added to the gel-shift reaction after preincubation of the probe and nuclear proteins on ice. Cold competition studies used a 200-fold excess of the unlabelled oligonucleotide. Band intensities were quantified as mean area density using ImageQuant (Molecular Dynamics, Sunnyvale, California, USA).

Immunohistochemistry

The entire caecum and proximal colon from wild-type and IL10-null mice were dissected longitudinally, fixed in 10% neutral buffered formalin, sectioned into 5 μ m samples, and stained with haematoxylin and eosin (H&E) for light microscopic examination. Meanwhile, deparaffinised sections were incubated with primary antibodies as follows: mouse or rabbit anti-pSTAT5, rabbit anti-p65, GHR, CIS and PPAR γ . Sections were incubated with isotype control serum (Santa Cruz Biotechnology) or pre-immune sera (AL-47) alone in place of primary antibody as a negative control, or with the corresponding blocking peptide to identify the specificity of antibodies. After a 1-h incubation at room temperature, sections were washed with PBS. Streptavidin biotin peroxidase was added and incubated for 45 min at room temperature. Haematoxylin was used for nuclear counterstaining after peroxidase (DAB) development. Images were captured using a Zeiss microscope and Axioviewer image analysis software (Carl Zeiss Corporation, Jena, Germany).

RESULTS

Chronic administration of growth hormone activates STAT5b and increases expression of PPAR γ in colitis

We first asked whether chronic administration of growth hormone would up regulate STAT5 and PPAR γ in the normal or inflamed mouse colon. We treated IL10-deficient mice with established colitis and wild-type controls with growth hormone twice daily for 2 weeks, and determined the effect on activation of STAT5 and expression of PPAR γ mRNA. We found that nuclear abundance of STAT5b was markedly up regulated by growth hormone in both IL10-deficient mice and wild-type mice, although the relative up regulation in wild-type controls was greater (fig 1A). The mean (standard deviation (SD)) nuclear abundance of pSTAT5 increased from 5 (1) to 29 (8) relative units after administration of growth hormone to wild-type mice, as compared with an increase from 28 (5) to 64 (9) relative units in IL10-deficient mice. Interestingly, activation of STAT5 was constitutively increased in IL10-deficient mice; immunohistochemistry (IHC) showed that this was largely confined to the lamina propria mononuclear cells (LPMC), with some expression in the epithelial layer (fig 1B). After administration of growth hormone, an increased frequency of pSTAT5-positive CEC and LPMC was observed in the colon of IL10-deficient mice (fig 1B). Concomitant with activation of STAT5b, expression of PPAR γ mRNA was up regulated in both wild-type and colitic IL10-deficient mice after chronic growth hormone treatment (fig 1C). As with the activation of STAT5b, the relative up regulation of expression of PPAR γ was greater in the wild-type mice (from 1 (1) to 2 (1.7) relative units) than in the IL10-deficient mice (from 1 (1) to 1.5 (0.9) relative units).

Administration of growth hormone reduces p65 activation in colitis and in TNF α -treated CEC in culture

As both STAT5b and PPAR γ can reduce activation of NF κ B, we asked whether administration of growth hormone regulated nuclear abundance of the NF κ B p65 subunit in colitis. The nuclear abundance of p65 was increased in the colon of

IL10-deficient mice, relative to wild-type controls, from 11 (3) to 44 (22) relative units. As fig 2A shows, chronic administration of growth hormone significantly reduced nuclear abundance of p65 in the colon of both wild-type and IL10-deficient mice, to 3.2 (3) and 25 (14) relative units, respectively. As we recently reported, this led to a significant reduction in mucosal inflammation.⁷ By comparison, administering a single dose of growth hormone for 30 min did not reduce nuclear abundance of p65 in the colon of IL10-deficient mice (fig 2B).

It was important to determine whether growth hormone could directly inhibit TNF α -dependent activation of p65 in CEC. We therefore determined the effect of growth hormone on nuclear abundance of p65 in T84 cells that had been pretreated with TNF α . As fig 3 shows, growth hormone alone rapidly induced tyrosine phosphorylation of STAT5. TNF α alone increased nuclear abundance of p65, while reducing cytosolic abundance of the GHR. TNF α pretreatment partially inhibited activation of STAT5 by growth hormone. In contrast with the colitic mice, short-term growth hormone treatment of T84 cells after exposure to TNF α significantly reduced nuclear abundance of p65. Concurrent with this, cytosolic abundance of the GHR and the inhibitors of activation of NF κ B, I κ B α and I κ B β were increased (fig 3B).

Neutralisation of TNF α up regulates the colon GHR:STAT5b:PPAR γ pathway

As we previously found that TNF α blockade would rapidly up regulate liver growth hormone signalling in IL10-deficient mice, we determined whether this would also induce the GHR:STAT5:PPAR γ pathway in the colon. We administered a single dose of an anti-TNF α antibody or isotype control (1 mg intraperitoneal) to IL10-deficient mice with established colitis, and prepared tissue sections and nuclear and cytosolic proteins of the colon 24 h later. As fig 4A shows, TNF α neutralisation

rapidly increased cytosolic GHR abundance in the colon, from 29.9 (7.5) relative units in isotype-treated controls to 47.4 (12.3) relative units by 24 h after administration of anti-TNF α (fig 4A). IHC localised this increase primarily to the CEC, with some expression of GHR also detected in the LPMC (fig 4B).

EMSA has shown that administering a single dose of growth hormone (1 μ g/g intraperitoneal 30 min before killing) significantly increased STAT5 DNA binding in normal mouse colon, from 13.3 (4.1) to 39.5 (13) relative units (fig 5A). By comparison, STAT5 DNA-binding activity was constitutively increased in IL10-deficient mice with colitis; this did not change with single doses of growth hormone. Supershift confirmed that the shifted complex contained both STAT5a and STAT5b (fig 5B). Western blot and EMSA using nuclear proteins showed that neutralisation of TNF α induced a significant increase in both activated STAT5 (pSTAT5) and nuclear abundance of PPAR γ , and in STAT5 DNA binding. This corresponded to an increase from 29.6 (7) to 64.2 (15.4) relative units for pSTAT5, from 27.3 (6.2) to 71.4 (9) relative units for PPAR γ and from 38.9 (9) to 65.6 (6) relative units for STAT5 DNA binding (fig 5B). A single dose of growth hormone given 30 min before killing did not further enhance activation of STAT5, relative to the effect of TNF α neutralisation alone (fig 5C). IHC localised the increases in pSTAT5 and the STAT5 target genes CIS and PPAR γ to the CEC, mimicking the localisation of the increases in GHR (fig 6). We then determined whether the rapid increase in nuclear abundance of pSTAT5 and PPAR γ in the colon after neutralisation of TNF α led to a significant decrease in activation of NF κ B.

Neutralisation of TNF α reduces activation of NF κ B

Nuclear abundance of p65 in the colon was significantly reduced within 24 h of administration of anti-TNF α antibody, relative to the isotype control (fig 7A). This corresponded to

Figure 3 Growth hormone (GH) reduces nuclear abundance of p65 in tumour necrosis factor α (TNF α)-treated T84 cells. T84 cells were treated with TNF α (100 ng/ml for 12 h) or human growth hormone; (500 ng/ml for 30 min), and nuclear (A) and cytosolic (B) proteins were prepared. Western blots were performed as shown. Results representative of four independent experiments are shown. NE, nuclear extracts; CE, cytosolic extracts. Signal intensity was determined by densitometry and is shown as mean (SD), n=4.

Figure 4 Neutralisation of tumour necrosis factor α (TNF α) increases abundance of growth hormone receptor (GHR) in colitis. Mice with established colitis due to interleukin 10 (IL10) deficiency and wild-type (WT) controls were treated with anti-TNF α antibody or isotype control (1 mg intraperitoneal 24 h before killing), and cytosolic proteins of the colon were prepared. IgG: isotype control antibody treatment. (A) Abundance of GHR and actin were determined by western blot. Signal intensity was determined by densitometry and is shown as mean (SD), $n = 4$. (B) Immunohistochemistry was performed to localise changes in expression of GHR. Results representative of six cases are shown. Original magnification $\times 400$, bar = 50 μ m, inset is negative control.

63.4 (10.3) relative units in isotype-treated controls versus 39.5 (10.1) relative units after neutralisation of TNF α . This was in contrast to a single dose of growth hormone, which did not reduce nuclear abundance of p65 in vivo (fig 2B). Western blot using cytosolic proteins showed that neutralisation of TNF α led to a marked increase in the abundance of I κ B α and I κ B β relative to isotype-treated controls, and therefore redistributed these cellular inhibitors of NF κ B (fig 7B). As recently reported, we subsequently observed a considerable improvement in histology and feed efficiency (weight gain normalised to chow intake) of the colon in IL10-deficient mice which had received the anti-TNF α antibody weekly for 2 weeks.⁵ Taken together, these data show that neutralisation of TNF α would rapidly (within 24 h) increase a novel anti-inflammatory GHR:STAT5b:PPAR γ pathway in an affected colon, and reduce activation of the p65 subunit of the pro-inflammatory NF κ B transcription factor.

DISCUSSION

Crohn's disease is becoming increasingly common in North America, with approximately 1 000 000 people affected.³⁰ Recent genetic studies have implicated primary defects in mucosal innate immunity, which lead to chronic, relapsing and remitting T cell predominate gut inflammation.³¹ Despite these

advances in our understanding of the disease pathogenesis, many children continue to experience growth failure and ongoing mucosal inflammation with current treatment.¹⁻³² We asked whether the growth hormone-dependent STAT5 transcription factor, which we recently linked to growth failure in colitis, might also regulate mucosal inflammation.⁵⁻³³ In this study, we identified a novel anti-inflammatory STAT5-dependent pathway in the colon, which can be up regulated via either chronic administration of growth hormone or neutralisation of TNF α .

Targeted deletion of the GHR has recently identified PPAR γ as a growth hormone target gene, and growth hormone has been shown to regulate the human PPAR γ 3 gene promoter via activation of STAT5b.¹²⁻³⁴ The GHR is expressed on small bowel cells, and CEC and resident macrophages.¹⁰ However, it was not known whether growth hormone would activate STAT5b and up regulate expression of PPAR γ in the gut. We found that growth hormone increased nuclear abundance of STAT5b in the normal mouse colon. Consistent with this, chronic administration of growth hormone led to a twofold increase in expression of PPAR γ . As we have recently shown that activation of STAT5b and IGF-1 by growth hormone is reduced in the liver in murine colitis, we asked whether the growth hormone:STAT5b:PPAR γ pathway would be reduced in inflamed colon.⁵

A

B

C

Figure 5 Neutralisation of tumour necrosis factor α (TNF α) increases activation of STAT5 and abundance of (PPAR γ) in colon epithelial cells (CEC). Mice with established colitis due to interleukin 10 (IL10) deficiency and wild-type controls were treated with anti-TNF α antibody or isotype control (1 mg intraperitoneal 24 h before killing) or human growth hormone (GH; 1 μ g/g intraperitoneal 30 min before killing) and nuclear and cytosolic proteins of the colon were prepared. (A, B) STAT5 DNA binding was determined by electrophoretic mobility shift assay. (A) Supershift for STAT5a and STAT5b was performed. Signal intensity was determined by densitometry and is shown as mean (SD), $n = 4$. (B) Nuclear abundance of pSTAT5 and PPAR γ as determined by western blot. Signal intensity was determined by densitometry and is shown as mean (SD), $n = 4$. (C) Mice were treated with anti-TNF α antibody or isotype control (1 mg intraperitoneal) for 24 h and also treated with rat growth hormone 1 μ g/gm intraperitoneal 30 min before killing. Nuclear proteins of the colon were prepared, and nuclear abundance of pSTAT5 and SHP1 was determined by western blot.

Figure 6 Immunohistochemistry (IHC) was performed to localise changes in abundance of pSTAT5/CIS/PPAR γ . Results representative of six cases are shown. Original magnification $\times 400$, bar = 50 μ m. IL10, interleukin 10; TNF, tumour necrosis factor.

Activation of STAT5b and up regulation of PPAR γ by growth hormone were relatively reduced in the colon of IL10-deficient mice, identifying for the first time a local resistance to growth hormone in the inflamed colon. This was consistent with the resistance to systemic (primarily, liver) growth hormone function in Crohn's disease in humans and in murine colitis, which we and others have recently described.^{1 2 5 35} This probably represents a secondary effect due to local release of inflammatory cytokines, including TNF α , rather than a primary defect, as administration of an anti-TNF α antibody restores growth hormone signalling in the liver within 24 h in both Crohn's disease and murine colitis due to IL10 deficiency.^{2 5} However, it is important to note that chronic administration of growth hormone was able to partially overcome this, leading to important anti-inflammatory effects, including down regulation of nuclear abundance of p65. By comparison, we found that neutralisation of TNF α rapidly increased activation of GHR, STAT5b and PPAR γ or abundance in CEC, within 24 h of the first dose of an anti-TNF α antibody, but down regulated nuclear abundance of p65 to the same degree. This led to an improvement in mucosal inflammation with chronic administration of anti-TNF α antibody.⁵

It is important to note that STAT5 was constitutively activated in LPMC of the colon in IL10-deficient mice. This may be due to cytokines, including IL2 and granulocyte-macrophage colony-stimulating factor (GM-CSF), which activate STAT5 in lymphocytes and macrophages, respectively. The functional significance of this is not currently known, as STAT5

may regulate both effector and regulatory T cells in the gut. Ongoing studies will determine the immuno-phenotype of the pSTAT5-positive LPMC in IL10-deficient mice, and will determine whether these have a predominately pro-inflammatory or anti-inflammatory function. However, our data suggest that the predominant anti-inflammatory effect of growth hormone or administration of anti-TNF in this regard involves activation of STAT5 in CEC, leading to increased expression of PPAR γ and reduced activation of NF κ B in the epithelium. This may reduce mucosal inflammation by decreasing epithelial expression of NF κ B-dependent pro-inflammatory mediators including chemokines.

It has not previously been proposed that a defect in activation of growth hormone-dependent STAT5 may contribute to impaired mucosal tolerance in inflammatory bowel disease. We have now shown that a novel anti-inflammatory GHR:STAT5:PPAR γ pathway may be up regulated in the inflamed colon via either chronic administration of growth hormone or neutralisation of TNF α . This mechanism may partly account for the rapid mucosal healing (via increased PPAR γ and reduced NF κ B), which has now been described in patients with Crohn's disease treated with infliximab.³² It is important to note that both growth hormone and GM-CSF, which can also directly activate STAT5, have shown beneficial effects in early clinical and pre-clinical studies in Crohn's disease.^{7 8 36-38} This raises the possibility that combination therapies (eg, neutralisation of TNF α and administration of growth hormone or GM-CSF) might act in a synergistic fashion to augment

Figure 7 Neutralisation of tumour necrosis factor α (TNF α) reduces nuclear abundance of p65 and increases cytosolic abundance of I κ B α in colitis. Mice with established colitis due to interleukin 10 (IL10) deficiency and wild-type controls were treated with growth hormone (GH; 1 μ g/g 30 min before killing) or anti-TNF α antibody (1 mg intraperitoneal 24 h before killing) and cytosolic and nuclear proteins of the colon were prepared. IgG: isotype control antibody treatment. p65 and SHP1 (A) and I κ B α , I κ B β and actin abundance (B) were determined by western blot. Signal intensity was determined by densitometry and is shown as mean (SD), n = 4.

intestinal activation of STAT5 and reduce mucosal inflammation.

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