Interleukin-10-independent anti-inflammatory actions of glucagon-like peptide 2

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Ivory CPA, Wallace LE, McCafferty D, Sigalet DL. Interleukin-10-independent anti-inflammatory actions of glucagon-like peptide 2. Am J Physiol Gastrointest Liver Physiol 295: G1202–G1210, 2008. First published October 9, 2008; doi:10.1152/ajpgi.90494.2008.—Glucagon-like peptide 2 (GLP-2) is an important intestinal growth factor with anti-inflammatory activity. We hypothesized that GLP-2 decreases mucosal inflammation and the associated increased epithelial proliferation by downregulation of Th1 cytokines attributable to reprogramming of lamina propria immune regulatory cells via an interleukin-10 (IL-10)-independent pathway. The effects of GLP-2 treatment were studied using the IL-10-deficient (IL-10−/−) mouse model of colitis. Wild-type and IL-10−/− mice received saline or GLP-2 (50 μg/kg sc) treatment for 5 days. GLP-2 treatment resulted in significant amelioration of animal weight loss and reduced intestinal inflammation as assessed by histopathology and myeloperoxidase levels compared with saline-treated animals. In colitis animals, GLP-2 treatment also reduced crypt cell proliferation and crypt cell apoptosis. Proinflammatory (IL-1β, TNF-α, IFN-γ) cytokine protein levels were significantly reduced after GLP-2 treatment, whereas IL-4 was significantly increased and IL-6 production was unchanged. Fluorescence-activated cell sorting analysis of lamina propria cells demonstrated a decrease in the CD4+ T cell population following GLP-2 treatment in colitic mice and an increase in CD11b+/F4/80+ macrophages but no change in CD25+FoxP3+ T cells or CD11c+ dendritic cells. In colitis animals, intracellular cytokine analysis demonstrated that GLP-2 decreased lamina propria macrophage TNF-α production but increased IGF-1 production, whereas transforming growth factor-β was unchanged. GLP-2-mediated reduction of crypt cell proliferation was associated with an increase in intestinal epithelial cell suppressor of cytokine signaling (SOCS)-3 expression and reduced STAT-3 signaling. This study shows that the anti-inflammatory effects of GLP-2 are IL-10 independent and that GLP-2 alters the mucosal response of inflamed intestinal epithelial cells and macrophages. In addition, the suggested mechanism of the reduction in inflammation-induced proliferation is attributable to GLP-2 activation of the SOCS-3 pathway, which antagonizes the IL-6-mediated increase in STAT-3 signaling.

lamina propria; crypt cell proliferation; interleukin-6; suppressor of cytokine signaling-3; signal transducer and activator of transcription-3

GLP-2 induces crypt cell proliferation and nutrient transporter expression, presumably to increase the absorptive capacity of the intestine (21, 28). These actions are mediated through the GLP-2 receptor, which is expressed on enteric neurons, myofibroblasts, and enteroendocrine cells (15, 37). In addition to these trophic effects, we have recently demonstrated that GLP-2 has significant anti-inflammatory actions in intestinal mucosa; in parallel, GLP-2 causes a normalization of the elevated rate of crypt cell division in inflamed mucosa to normal levels (24). These data suggest that the anti-inflammatory effects of GLP-2 may be mediated by pathways that are distinct from the trophic effects.

In our previous studies, we showed that GLP-2 treatment speeds mucosal healing, reducing mucosal inflammation and the local expression of inflammatory cytokine production (IL-1β, IFN-γ, and TNF-α) through the activation of enteric vasoactive intestinal peptide (VIP)-expressing neurons (24). Previous workers have proposed that activation of VIP neurons leads to a predominately Th2 anti-inflammatory response (1, 9). In multiple models of inflammation, VIP has been shown to increase the production of IL-4 and IL-10 (1, 13, 23). In intestinal models of inflammation, VIP modulates the inflammatory activity of lamina propria macrophages, CD4+ lymphocytes, and dendritic cells, leading to a suppression of inflammatory cytokine output and improved healing (IL-1β, TNF-α, IFN-γ, and IL-6) (1, 9, 14). Taken together, these studies suggest that GLP-2 modulates intestinal inflammation via activation of VIP-expressing neurons, and this would be expected to act via an increase in Th2 cytokines such as IL-10 and IL-4. However, in our previous study in which GLP-2 had clear anti-inflammatory effects in two different models of colitis, we observed differing effects on IL-10 production. Specifically, there was no increase in mucosal IL-10 production in the trinitrobenzene sulfonic acid (TNBS) colitis model (31). These data suggest that the anti-inflammatory actions of GLP-2 are not mediated through IL-10.

In this study, we examined the requirement for IL-10 production in the anti-inflammatory activity of GLP-2 by examining the effects of exogenous GLP-2 in the IL-10-deficient (IL-10−/−) model of colitis. In addition, we examined the effects of GLP-2 on the activity of the immunoregulatory cells of the lamina propria to determine the pathways it may be acting through. Finally, we examined the mechanisms underlying the reduced proliferation of epithelial cells described in the specific context of GLP-2-treated intestinal inflammation. Elevated levels of IL-6 have been associated with crypt cell proliferation and nutrient transporter expression.
hyperproliferation in experimental models of colitis (27); we hypothesized that a reduction in mucosal IL-6 is the mechanism by which GLP-2 normalizes crypt cell proliferation.

We show that GLP-2 administration ameliorates colitis in IL-10−/− mice and that this was associated with a reduction of TH1 inflammatory cytokines. GLP-2 increased the lamina propria macrophage population and induced macrophage IGF-1 production but did not increase mucosal transforming growth factor (TGF-β) production. Consistent with our previous report, GLP-2 reduced crypt cell proliferation in inflamed tissue, but there was no change in IL-6 levels. However, this effect was associated with an increase in SOCS-3 mRNA in intestinal epithelial cells and downregulation of IL-6 STAT-3 signaling. These findings contribute to our understanding of the mechanisms of GLP-2 action on intestinal inflammatory response and healing.

MATERIALS AND METHODS

Experimental plan. The general effects of GLP-2 treatment on IL-10−/−-associated colitis were first assessed by treating IL-10−/− or background 129SvEv animals with GLP-2 or saline, with the endpoints of general animal well-being, colonic inflammation, inflammatory cytokine production, and epithelial cell kinetics. Having determined that GLP-2 treatment was effective in this model, and thereby not dependent on IL-10 production, we then examined the effects on specific subpopulations of lamina propria immune cells and their cytokine profile. We next examined the mechanism of the reduction in the inflammation-induced increase in crypt cell proliferation by probing the activity of the IL-6 and suppressor of cytokine signaling (SOCS)-3 regulators of STAT-3 signaling.

Animals and reagents. IL-10−/− and control 129SvEv male mice were bred on site at the University of Calgary and used at 3 mo of age for all studies performed. All animal protocols were approved by the University of Calgary Animal Care Committee following the guidelines of the Canadian Council on Animal Care. To study the effects of GLP-2 on colitis, animals were treated with saline or GLP-2 (50 μg/kg per day, given BID, 0.2 ml sc). GLP-2 [human recombinant (1–33)] was a generous gift from NPS Pharmaceuticals (Mississauga, ON, Canada).

Tissue sampling and histology. At euthanasia, animals received the normal morning injection of GLP-2 or saline. One hour later, bromodeoxyuridine (BrDU) was injected (120 mg/kg ip; Sigma, St. Louis, MO). One hour after BrDU injection, animals were euthanized. Segments of colon (distal 7 cm) were resected and flushed with 5 ml of iced saline. The proximal 2 cm were fixed (10% formalin) and processed for regular histology or immunohistochemical studies. Mucosal morphology (inflammation scoring) was measured using previously described techniques (2, 21). Inflammation was rated on a 0–3 scale, reviewing all areas of mucosa, both intact and ulcerated zones (2). Similarly, from areas of intact mucosa, BrDU-specific labeling was used to quantify dividing crypt cells and activated caspase-3 immunostaining for detection of apoptotic crypt cells in 10-well oriented crypt units; in each crypt column, total cells were also counted (8, 31). Values are reported as BrDU (antibody: 1:100 rabbit anti-BrDU; Serotec, London, UK) or activated caspase-3 (1:100 rabbit anti-activated caspase-3 antibody; BD Pharmingen, San Diego, CA) labeled cells per crypt column divided by the total cells per crypt column (crypt proliferation or apoptotic index, respectively). The next 2 cm of specimen were frozen at −80°C for myeloperoxidase (MPO) assay (24), and the remaining mucosa was scraped and frozen for cytokine assays.

Cytokine ELISA analysis. IL-1β, IFN-γ, TNF-α, IL-4, and TGF-β content in mucosal scrapings were determined with the use of commercially available mouse-specific ELISA kits following the manufacturer’s instructions (cytokine kits from BioSource, Camarillo, CA).

Isolation of lamina propria cells. Suspensions of lamina propria cells were prepared using established techniques (10). Briefly, after mechanical cleansing, the colonic sections were cut longitudinally and into 5-mm pieces, washed in HBSS (calcium and magnesium free), and treated four times with 5 mM EDTA (Sigma) at 37°C for 15 min and then rinsed in HBSS. Loss of epithelial cells was confirmed by light microscopy. Remaining tissue was digested using 100 U/ml collagenase type II (Invitrogen, Carlsbad, CA) in HBSS at 37°C for 1 h. Supernatants were collected and tissue pieces redigested using the same conditions until no tissue was observed. The supernatants were washed with fresh RPMI-1640 (Invitrogen), spun at 1,200 revolution/min, and the pellet resuspended in PBS. Cell viability was assessed at 95% with propidium iodide stain. The purity of the cell preparations was confirmed by flow cytometric analysis of the epithelial cell fractions: less than 0.3% of cells released from the precollagenase digests stained positively for immune cell markers (CD-4, CD-8, CD-11b, F4/80) (data not shown). The use of flow cytometry ensured that only cells staining for immune-specific antigens released during the collagenase extraction phase were analyzed.

Flow cytometry. Isolated cells were incubated with various PE-, or FITC-labeled mAbs (eBioscience, San Diego, CA), fixed in 0.4% paraformaldehyde and analyzed on a FACScan flow cytometer (Becton Dickinson, San Jose, CA). For analysis of cytokine production, isolated cells were first activated with leukocyte activation cocktail (BD Pharmingen) for 4 h at 37°C and subsequently fixed and stained using the intracellular cytokine staining kit (BD Pharmingen). Antibodies against regulatory peptides (IGF-1, TGF-β) were purchased form Santa Cruz Biotechnology (Santa Cruz, CA). All flow cytometric analyses were performed with Cell Quest Pro.

Real-time PCR. Cells were isolated from the lamina propria as described above, and mRNA was isolated using TRIzol Reagent (Invitrogen). A sample (1 μg) of total mRNA was reverse transcribed with Superscript II enzyme (Invitrogen) and random hexamer primers (Amersham, Arlington Heights, IL). Real-time PCR analysis using ExpressSybGreenER (Invitrogen) and mouse-specific primers were used to determine relative cytokine gene expression. Cytokine gene expression was normalized to L32 ribosomal RNA and is represented as fold increase over control. DNA amplification assays were conducted in triplicates with the 7900HT fast real-time PCR machine (Applied Biosystems, Foster City, CA).

SOCS-3 expression and STAT-3 Western blot. Intestinal epithelia cells were isolated from the colon for mRNA. Briefly, colons were washed in HBSS (calcium and magnesium free), sectioned, and treated with 1 mM EDTA (Sigma) at 37°C for 15 min. Colonic epithelial cells in the supernatant were recovered by gentle centrifugation. Cell pellets were used to extract mRNA using TRIzol Reagent (Invitrogen). SOCS-3 mRNA expression (27) was assessed by real-time PCR as described above. Mucosal scrapings were used for Western blotting. Protein extracts were resolved by 12% SDS-PAGE and transferred onto polyvinylidene difluoride membranes. Immunoblotting was performed using anti-phosphorylated STAT3 (pY-STAT3) or SOCS-3 (Santa Cruz Biotechnology) and anti-β-actin (Sigma) primary antibodies. Bound antibody was visualized using secondary horseradish peroxidase-conjugated antibody and enhanced chemiluminescence (Lumi-light, Roche Diagnostics, Indianapolis, IN).

RESULTS

Effects of GLP-2 on intestinal inflammation. The global effects of GLP-2 in the IL-10−/− model of colitis or in wild-type control animals were assessed following 5 days of treatment with saline or GLP-2. All animals lost some weight.
during the 5-day study period; however, there was no significant difference between wild-type mice given saline or GLP-2 (Fig. 1A). The IL-10-deficient mice treated with GLP-2 had significantly less weight loss than IL-10−/− mice treated with saline alone (P < 0.05). In parallel, GLP-2 treatment significantly reduced the inflammation scores of colitis in IL-10−/− mice (Fig. 2). GLP-2 also significantly reduced MPO levels in IL-10−/− mice, whereas baseline levels were similar in all wild-type animals (Fig. 1B). Histological examination of colonic tissues revealed a reduction in crypt cell proliferation (Fig. 1C) in GLP-2-treated IL-10−/− mice, consistent with our previous reports (31). This reduction was
significant compared with the proliferation rates determined by BrdU staining in saline-treated inflamed IL-1$^{-/-}$ mice. As expected, GLP-2 treatment in wild-type mice induced a significant increase in cell proliferation, consistent with previous reports of GLP-2 effects on noninflamed tissue (11). GLP-2 treatment also reduced crypt cell apoptosis, as measured by activated caspase-3 staining in colonic sections (Fig. 1D). Saline-treated IL-10$^{-/-}$ mice showed alterations in mucosal architecture, whereas GLP-2 restored a normal mucosal structure comparable to wild-type colonic sections (see Fig. 2).

GLP-2 treatment reduces inflammatory cytokines. IL-10$^{-/-}$ mice spontaneously develop chronic colitis, characterized by elevated levels of IL-1β, IL-6, and TNF-α (29). The anti-inflammatory effects of GLP-2 in the IL-10 model of colitis have not been examined to date, and, therefore, changes in cytokine production were measured. There were marked differences in proinflammatory cytokines between saline- and GLP-2-treated IL-10$^{-/-}$ mice. No significant differences were observed between wild-type animal groups. GLP-2 treatment significantly decreased IFN-γ, TNF-α, and IL-1β protein production and increased IL-4 cytokine production (Fig. 3), whereas TGF-β and IL-6 levels were not affected. To elucidate the source of the inflammatory cytokines noted above, lamina propria immune cells from the colon (CD4$^+$, CD11c$^+$, and F4/80$^+$ subpopulations by flow cytometry) were analyzed using real-time PCR for cytokine mRNA, and identical findings were noted (data not shown).

Effects of GLP-2 on lamina propria cells. We investigated whether GLP-2 treatment altered immune cell populations, which could account for the dampening of inflammation. Lamina propria cells were isolated from mice treated with saline or GLP-2 and stained for flow cytometry. Interestingly, at the 5-day mark, we did not observe significant differences in the CD4$^+$ T cell population between wild-type (22.6%) and IL-10$^{-/-}$ (22.2%) saline-treated mice (Fig. 4A). However, GLP-2 treatment reduced CD4$^+$ T cell numbers in both wild-type (14.2%) and IL-10$^{-/-}$ mice (7.6%), and this effect was greater in inflamed animals (Fig. 4A). We noted the opposite pattern with the unique CD11b$^+/F4/80^+/CD11c^-$ lamina propria macrophage population, with GLP-2 treatment causing a significant increase in these cells (Fig. 4B). Saline-treated IL-10$^{-/-}$ mice had a greater lamina propria macrophage pop-
ulation (37%) than matched wild-type controls (26%). Five-
day treatment with GLP-2 caused a further increase in lamina
propria F4/80 macrophages in both IL-10−/− mice treated with saline or
GLP-2; GLP-2 treatment significantly reduced the number of CD3/CD4 positive cells in the lamina
propria of IL-10−/− mice. B: CD11b/F4/80 positive macrophages isolated from the lamina propria;
GLP-2 treatment significantly increases the numbers of CD11b/F4/80 macrophages in the lamina propria
of IL-10−/− mice. Numbers in the upper right cor-
ner indicate percent positive cells. Data are repre-
sentative of 3 or more independent experiments;
*P < 0.05.

Fig. 4. GLP-2 effects on lamina propria cell popu-
lations. Animals were treated daily with saline or
GLP-2 for 5 days (n = 8/group), and lamina propria
cells were isolated for flow cytometry. A: number of
CD3/CD4 positive T cells in the lamina propria of
wild-type and IL-10−/− mice treated with saline or
GLP-2; GLP-2 treatment significantly reduced the
number of CD3/CD4 positive cells in the lamina
propria of IL-10−/− mice. B: CD11b/F4/80 positive macrophages isolated from the lamina propria;
GLP-2 treatment significantly increases the numbers
of CD11b/F4/80 macrophages in the lamina propria
of IL-10−/− mice. Numbers in the upper right cor-
ner indicate percent positive cells. Data are repre-
sentative of 3 or more independent experiments;
*P < 0.05.
keeping with the ELISA results, we also did not observe an increase in TGF-β in FoxP3+ T regulatory positive lamina propria cells isolated from GLP-2-treated mice (Fig. 5D); the proportion of FoxP3+ T cells expressing TGF-β levels were also similar in both saline-treated (40%) and GLP-2-treated (35%) control mice although GLP-2 did cause a significant reduction in mucosal TGF-β production in control animals (Fig. 3).

**Effects of GLP-2 on crypt cell proliferation.** In our previous study, we observed that GLP-2 could effectively reduce crypt...
cell proliferation in rat models of TNBS and dextran sulfate sodium colitis (31). This finding was remarkable given that GLP-2 is reported to induce crypt cell proliferation in other situations, such as experimental short bowel syndrome (21, 22). Because chronic inflammation is known to induce cytokines such as IL-6 that promote crypt cell proliferation, we probed the mechanisms underlying GLP-2 antiproliferation activity in this model. First, we determined IL-6 expression in lamina propria immune cells (by intracellular detection using flow cytometry) in saline- and GLP-2-treated IL-10−/− mice. We did not detect any significant changes in IL-6 expression after GLP-2 treatment (Fig. 6A). We next assessed intestinal epithelial expression of SOCS-3, which is known to limit damage induced crypt cell proliferation (27). Real-time PCR analysis of intestinal epithelial cell mRNA demonstrated an increase in SOCS-3 expression in GLP-2-treated IL-10−/− compared with controls (Fig. 6B). This increased mRNA expression corresponded to an increase in SOCS-3 protein in mucosal scrapings from GLP-2-treated IL-10−/− mice (Fig. 6C). Because IL-6 levels were not decreased, we determined whether cytokine signaling is altered with SOCS-3-increased expression in these cells. Western blot analysis for phosphorylated STAT-3 in mucosal scrapings showed increased STAT-3 activation in inflamed saline-treated IL-10−/− mice, whereas GLP-2 treatment reduced this STAT-3 activation (Fig. 6D).

**DISCUSSION**

These results demonstrate that administration of exogenous GLP-2 can ameliorate chronic colitis in IL-10-deficient mice. Resolution of disease was associated with a decrease in proinflammatory cytokines and a reduction in crypt cell proliferation and apoptosis. These results show that GLP-2 effects were not dependent on IL-10 and that alternative immune regulatory mechanisms are involved.

The IL-10 knockout mouse model of colitis is characterized by an infiltration of mononuclear cells producing colonic lesions at ~3 mo of age (26). The resulting colitis is sustained by an increase in IL-1β, IL-6, TNF-α, and IFN-γ from Th1 cells. The role of IL-10 is described as preventative because administration of recombinant IL-10 to adult IL-10−/− mice does not cure colitis. Thus exogenous GLP-2 appears to be acting in part to eliminate Th1 cells or block their effector functions. In this study, as anticipated, the reduced inflammation seen with GLP-2 treatment was associated with a decrease in IL-1β, TNF-α, and IFN-γ in the local inflammatory milieu. Additionally, there was a significant decrease in the proportion of activated CD4+ lymphocytes (Fig. 4). One possible explanation for these findings would be a GLP-2-mediated activation of alternative Th2 pathways, and, indeed, IL-4 production increased in lamina propria cells in GLP-2-treated mice (Fig. 3). However, since we did not detect an increase in other Th2 cytokines (IL-13 and IL-5, data not shown), we attributed the increase in this cytokine to the decrease in IFN-γ, which can antagonize the production of IL-4 (33). These findings extend those of our previous study in which we demonstrated no significant change in IL-10 in the colon of GLP-2-treated TNBS rats, which otherwise had reduced proinflammatory cytokine levels (31). The reduction (50%) in the proportion of activated CD4+ lymphocytes is significant since these are numerically the largest body of immune-competent cells in the

Fig. 6. GLP-2 effects on crypt cell proliferation signals. Animals were treated daily with saline or GLP-2 (n = 8/group), and lamina propria or intestinal epithelial cells were isolated. A: at day 5, intracellular levels of IL-6 in total lamina propria cells did not change with GLP-2 treatment in IL-10−/− mice (numbers in top left indicate cells positive for IL-6). B: suppressor of cytokine signaling 3 (SOCS-3) mRNA levels were determined by real-time PCR in epithelial cells isolated from saline- or GLP-2-treated wild-type or IL-10−/− mice (day 4). GLP-2 significantly increased (*P < 0.05) SOCS-3 expression in IL-10−/− mice. C: corresponding SOCS-3 protein expression in mucosal scrapings taken on day 5 from saline- or GLP-2-treated wild-type and IL-10−/− mice. D: Western immunoblotting on mucosal extracts from wild-type or IL-10−/− mice following saline or GLP-2 treatment showed increase phosphorylated STAT3 (pY-STAT3) in inflamed IL-10−/− mice. GLP-2 administration reduced pY-STAT3 levels in IL-10−/− mice. Data are representative of 3 or more independent experiments.
lamina propria, and this likely contributed to the reduction in inflammatory cytokine production (10) (Fig. 4). How then are these changes in the inflammatory activity of the intestinal mucosa occurring? Regulatory T cells, macrophages, and dendritic cells are all key regulators of inflammation in the lamina propria with demonstrated plasticity in their immune functions. Each of these regulatory cells can mediate the balance between inflammatory responses and tolerance (6, 10). Here we report that GLP-2 caused an increase in the number of lamina propria macrophages and a resetting of the signaling profile from inflammation to prohealing. This parallels the observations of previous studies, which showed that the lamina propria macrophage is uniquely able to downregulate its own inflammatory output and that of activated and naïve lymphocytes in the lamina propria (10, 32). Interestingly, our findings contrast with some aspects of this previous work, which suggested that the suppressor function of these macrophages was largely mediated by IL-10 (10). The present data suggest that this can occur in the absence of IL-10, which might implicate other cytokines or direct cellular signaling. In addition, we found a significant increase in IGF-1 production in these same lamina propria macrophages. The role of IGF-1 in intestinal inflammation and resolution has yet to be fully delineated, but there is large body of work that shows that it is increased, especially in the healing phases of chronic inflammation (18, 38). Systemic IGF-1 secretion is stimulated by growth hormone and mediates several developmental processes including proliferation, differentiation, survival, growth, and regeneration; however, in the intestine, local IGF-1 is produced by myofibroblasts associated with bowel growth and cell proliferation (8, 12). Previous studies have focused on the smooth muscle layers; the present data suggest that IGF-1 may also be important in the healing of mucosal lesions (18, 36). During colitis, the local production of IGF-1 may be pivotal in resolution of inflammation and disease outcome. As well, the local production of IGF binding proteins likely plays a role in IGF-1 activity; in a healthy gut, IGF binding protein-3 limits the bioavailability of IGF-1 and can regulate cell growth and apoptosis (17). The present studies are limited in that the levels of the IGF binding proteins were not determined; further studies will be required to investigate fully the effects of GLP-2 on the IGF-1 axis in the setting of inflammation.

In contrast to the significant changes in lamina propria CD-4⁺ lymphocytes and macrophages following GLP-2 treatment, we found no changes in the proportion of CD-11c⁺ dendritic cells, in the number of CD-25⁺ or CD-25⁺FoxP3⁺ cells, or in their production of inflammatory or regulatory proteins (Fig. 5). Specifically, we did not see an increase in TGF-β in either the mucosal protein extracts or in the proportion of FoxP3⁺ cells expressing TGF-β, suggesting that these were unlikely to be mediators of the anti-inflammatory effects of GLP-2 noted.

We demonstrate here and in previous work that GLP-2 reduced crypt cell proliferation in inflamed animals (31). Chronic inflammation induces an increase in crypt cell proliferation/regeneration in an attempt to repair mucosal damage (19). However, prolonged stimulation of proliferation has been linked to colitis-associated cancers (25). IL-6 has been described as one of the key cytokines responsible for hyperproliferation of crypt cells (5, 30). STAT-3 signaling has also been associated with cell survival and is highly activated in experimental models of inflammatory bowel disease (16, 34). IL-10 and IL-6 both signal via STAT-3, and IL-10 is known to balance the effects of IL-6. In the IL-10⁻/⁻ model, GLP-2 did not significantly decrease IL-6 production; however, GLP-2 treatment did reduce the rate of crypt cell proliferation associated with chronic inflammation (Figs. 1 and 6). We examined whether IL-6 signaling may have been reduced in GLP-2-treated IL-10⁻/⁻ mice by the specific action of SOCS-3. SOCS-3 has been reported to function as a factor limiting injury-induced crypt cell proliferation in the colon (7, 27) through inhibition of STAT-3 signaling by IL-6. In this study, we observed an increase in SOCS-3 and STAT-3 protein expression (despite modest changes in mRNA, similar to previous work examining SOCS3 and STAT-3 signaling in IL-10⁻/⁻ mice; see Ref. 34). Our study revealed that GLP-2 induced a dramatic increase in SOCS-3 expression and a significant reduction in STAT-3 activation in IL-10⁻/⁻ mice. These findings suggest that GLP-2 treatment reduces crypt cell proliferation in inflamed intestine by increasing SOCS-3 expression. Given that SOCS-3 has been shown to negatively regulate the formation of IL-17 secreting T cells and the activity of IL-23 in phosphorylating STAT-3, the relationships between these important regulatory systems and GLP-2 activity bears further direct investigation (4, 35). Specifically, in the context of chronic inflammation, these findings may be very important in understanding the development of inflammation-induced adenocarcinoma (19).

In summary, the results of this study have provided further insights into the mechanisms underlying the anti-inflammatory effects of GLP-2. We have determined that IL-10 and Th2 cytokines are not important in GLP-2 anti-inflammatory effects. Instead, we suggest a role for the lamina propria macrophage as a key mediator for GLP-2 activity in reducing proinflammatory cytokine production and increasing IGF-1 production. Moreover, we report a role for SOCS-3-induced reduction in STAT-3 signaling in GLP-2 reduction of crypt cell proliferation. As noted, GLP-2 receptors are not present on epithelial or immune cells, and the specific pathways through which GLP-2 signals are not clear. It is not known whether our previously postulated mechanism of GLP-2-induced release of VIP can induce the full range of immunological effects noted in this study; further studies are required. The delicate balance between chronic inflammation and proper repair mechanisms supports a role for GLP-2 in limiting damaging factors and promoting mucosal repair in the resolution of colitis. The unique linkage of these apparently profound immune regulatory effects and the previously described role for GLP-2 as an intestinal trophic hormone is important an area for study. There may be a link in the mechanisms underlying these effects or in the regulation of immune responses to enteral antigens.

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DISCLOSURES

Dr. Sigalet has acted as a paid consultant for NPS Pharmaceuticals (2007).

REFERENCES


