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Intestinal inflammation

Interleukin-6 is an important in vivo inhibitor of intestinal epithelial cell death in mice

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ABSTRACT

Background and aims Interleukin-6 (IL-6) is a well-recognised mediator of liver disease and regeneration. However, the in vivo effects of IL-6 on enterocytes and the intestinal tract have not been elucidated. We sought to determine the in vivo effects of IL-6 on enterocytes.

Methods Murine models of increased or absent IL-6 were examined.

Results Systemic, high-dose IL-6 administration to mice over 7–10 days resulted in intestinal hyperplasia with a ~40% increase in small bowel mass and in intestinal villus height. No increase in crypt cell proliferation was noted. IL-6 administration was associated with induction of pSTAT3 in enterocytes along the lower and middle regions of villi but not in crypts. IL-6 administration was also associated with induction of anti-apoptotic proteins including pAKT, and FLIP along with decreased executor caspase activity and PARP cleavage. Pulse bromodeoxyuridine labelling demonstrated equivalent crypt cell proliferation rates but prolonged enterocyte lifespan and slowed enterocyte migration rates in IL-6 treated mice. Furthermore, IL-6 treated mice showed less intestinal injury and improved barrier function following ischaemia reperfusion of the small bowel. Conversely, IL6 null mice exhibited impaired recovery following massive enterectomy and increased apoptosis after 5-fluorouracil chemotherapy relative to wild-type controls.

Conclusions IL-6 inhibited both constitutive and induced enterocyte cell death in vivo. Loss of IL-6 in mice resulted in increased activation of pro-apoptotic and necrotic pathways in enterocytes after injury. Therapies that augment IL-6 or its signalling pathways may help manage intestinal disorders associated with increased apoptosis, necrosis and gut injury.

INTRODUCTION

Acute intestinal injury is a common complication of many disease processes including the sequelae of elective surgery, trauma or shock. It can affect anyone at any age. Loss of intestinal barrier function from enterocyte disruption or cell death is a particularly devastating complication of acute intestinal injury that may lead to life-threatening bacterial translocation from the gut and numerous secondary organ injuries, multi-system organ failure and death. Many cytokines and growth factors are modulated in acute intestinal injury. These factors are involved in reparation and adaptation and include glucocorticoids, growth hormone, IGF-1, EGF, keratinocyte growth factor, leptin, ghrelin and GLP-2, as well as amino acids, sugars and fatty acids.1–6

In the liver, IL-6 is an acute, critical mediator of recovery following hepatectomy, Fas ligation, hepatitis and other liver injuries.7 Chronic IL-6 signalling predisposes hepatocytes to cell death and liver failure and chronic low levels of IL-6, as in obesity, contribute to hepatic insulin resistance.8–10 In the stomach, IL-6 has been implicated in gastric homeostasis.11 IL-6 is recognised as an important mediator of gut dysfunction in inflammatory bowel disease,12 and antibody neutralisation of IL-6 has been shown to improve gut function and lessen the severity of patient symptoms.12 In contrast to the large amount of research relating to the liver, stomach and inflammatory bowel disease, relatively little work has focused upon the physiological and potentially therapeutic roles of IL-6 in the intestinal response to surgical stress or other gut injury, particularly as it relates to cell death and proliferation. Given the different effects of IL-6 on different tissues and rapidly emerging IL-6 pathway related pharmaceuticals, understanding tissue-specific effects remains important. Therefore, we sought to identify the in vivo effects of IL-6 on enterocyte cell proliferation and death, as well as the effects of loss of IL-6 and exogenous IL-6 administration on intestinal homeostasis and repair.

MATERIALS AND METHODS

Reagents and antibodies

Reagents were purchased from Sigma (St Louis, Missouri, USA) unless otherwise specified. All other reagents were obtained from the following sources: recombinant murine IL-6 (PeproTech Inc, Rocky Hill, New Jersey, USA); SuperSignal West Pico or Femto Chemiluminescent Substrate, Coomassie Protein Assay (Pierce, Chicago, Illinois, USA); Bak (Upstate Biotechnology, Saratoga, Canada); Bak (Upstate Biotechnology, Santa Cruz, California, USA); Bak (Upstate Biotechnology, Albany, New York, USA); PCNA, β-actin, BrdU (Sigma); ImmunoPure peroxidase-conjugated secondary antibodies (Pierce); human and murine IL-6 Quantikine ELISA Kits (R&D Systems, Minneapolis, Minnesota, USA).

Cell lines and mouse studies

CHO IL-6 expressing and control cells were grown and used as previously reported.7–10 All mouse experiments were approved by the University of Miami Institutional Animal Care and Use Committee.
To measure enterocyte migration rates, C57BL/6J mice (Jackson Laboratories, Bar Harbor, Maine, USA) bowel were applied to prevent collateral previously reported in the rat, with the addition that small, non-crushing, vascular clamps on the superior and inferior small bowel were applied to prevent collateral flow. Fluorescein isocyanate (FITC)-labelled dextran was administered 5 min before ischaemia by gavage as described, and plasma was collected immediately after 60 min reperfusion. Wild-type C57BL/6J or homozygous null Il6 tm1Kopf (Jackson) underwent a 50% enterectomy, or as a control, transection and re-anastomosis of the small bowel as previously described. All operations and other in vivo procedures were carried out between 10 am and 1 pm under isoflurane general anaesthesia. Mice received 0.1 mg/kg buprenorphine intramuscularly for postoperative pain. Other mice were injected intra-peritoneally with 5-fluouracil (5-FU; 40 mg/kg body weight). For all analyses, mice were killed under general anaesthesia at reported time points or when clearly ill.

**TUNEL and BrdU labelling**

Terminal deoxynucleotidyl-transferase (TdT)-mediated dUTP nick-end labelling (TUNEL) staining was performed using the In Situ Cell Death Detection Kit (Roche, Indianapolis, Indiana, USA) on formalin-fixed, paraffin-embedded sections. Labelling of enterocytes with 5-bromo-2-deoxyuridine (BrdU) was done as previously described (40 mg/kg intraperitoneally 96 or 2 h prior to euthanasia). To measure enterocyte migration rates, double labelling of 5-BrdU was done, as previously described (4.8 mg/kg intraperitoneally at 48.5 h and 120 mg/kg intraperitoneally at 1.5 h prior to euthanasia). IL-6 induces gut hypertrophy in mice

Given that IL-6 is known to have pro-proliferative and anti-apoptotic effects on hepatocytes, we sought to define the in vivo effects of IL-6 on the intestinal tract. We previously examined the in vivo effects of IL-6 on the liver in nude mice injected intramuscularly with a CHO cell line transfected to express high concentrations of human IL-6. Using that model, administering IL-6 over 12 days resulted in intestinal tissue levels of 40–80 pg human IL-6/mg total protein (figure 1A). Sustained administration of IL-6 resulted in increased small bowel wet (figure 1B) and dry weights (data not shown) over time, peaking at ~152% of controls at days 9 and 12. Lengths of the small bowel were increased in IL-6 treated mice (relative length 1.24 ± 0.39 vs 1.00 ± 0.031, p < 0.001, n = 6 per group). Measurement of villi from histological sections revealed a ~42% lengthening of intestinal villi in IL-6 treated mice (398 ± 56.28 vs 278.9 ± 26.89 μm, p < 0.02) with no change in crypt depth (figure 1C). In contrast to effects on enterocytes, no increases in gut-associated lymphoid tissue or in thickness of the intestinal muscularis were observed (data not shown). Quantitation of crypt and villus cell numbers by counting nuclei in photographs of paraffin-embedded H&E-stained sections demonstrated a statistically significant increase in villus height and cell number, without a change in crypt cell depth (figure 1D). Despite increased villus length and small bowel weight in IL-6 treated mice, no increase in total PCNA expression by Western blotting of whole tissue lysates or in enterocytes by immunohistochemical staining was observed, suggesting that IL-6 induced intestinal hyperplasia was not associated with increased enterocyte proliferation (figure 1E,F and 2B).

To examine the possibility that IL-6 induced intestinal hyperplasia may be due to decreased cell death, we performed TUNEL staining on IL-6 treated and control duodenal, jejunal and colonic segments. Strikingly, ~52% fewer TUNEL-positive enterocytes were observed in sections from the small intestines in IL-6 treated mice versus controls (figure 2A). Western blotting analysis of whole small bowel extracts from IL-6 and control treated mice demonstrated a 40% reduction in cleaved caspase-3 (cCaspase-3) and a 75% reduction in its processed substrate, cleaved PARP (figure 2B). Taken together, these data suggest that IL-6 induced intestinal hyperplasia via a mechanism involving decreased enterocyte cell death and prolonged enterocyte lifespan without altered intestinal proliferation rates.

**Effects of IL-6 on proliferation and enterocyte lifespan in mice**

We next sought to determine directly the effect of IL-6 on enterocyte proliferation rates and enterocyte lifespan. Forty eight hours after injection with IL-6 or control cells, mice were injected with BrdU and euthanised 2 or 96 h later.
Formaldehyde-fixed, paraffin-embedded sections from the duodenum, jejunum and colon were subjected to BrdU immunohistochemical staining. Quantitation of BrdU positive nuclei at 2 h showed similar numbers of labelled crypt cells in the IL-6 treatment and control groups, suggesting that increased intestinal size and mass were not attributable to increased crypt cell proliferation (figure 1C,E). This was consistent with the indistinguishable expression of PCNA in IL-6 treated versus control mice (figure 2B). In contrast, analysis of sections of small intestine 96 h after BrdU injection demonstrated approximately threefold increased BrdU-positive cells in the villi of IL-6 treated intestines versus controls (figure 2D). Taken together, these results indicated that the same number of intestinal enterocytes were "born" in IL-6 treated mice, but that fewer labelled cells were lost or cells were lost more slowly than in controls. This persistence of labelled cells in IL-6 treated mice suggests that IL-6 administration prolonged enterocyte lifespan.

Enterocyte migration is a function both of cell proliferation in the crypts and cell death at the villus tip. To measure migration rates, we performed a dual-pulse BrdU experiment on control and IL-6 exposed mice. Forty eight hours after injection of IL-6 or control cells, mice were injected with a first dose of BrdU at 49.5 h hours prior to euthanasia, then a second dose 1.5 h before euthanasia. Because BrdU is rapidly cleared, this protocol results in pulse labelling only of cells born in the peri-labelling period and permits quantitative determination of cell proliferation and migration rates. Quantitation of BrdU-positive cells along the longitudinal villus axis demonstrated two peaks.
of labelling consistent with the two bolus injections of BrdU (figure 3A, B). Villi of the IL-6 treated mice were considerably longer owing to a greater number of cells, 108 \( \pm \) 6 vs 96 \( \pm \) 8.5 cells in controls (p < 0.02) (figure 3B). The calculated migration rate was \( \sim \) 15% slower in the IL-6 group than in the control group (figure 3C). Thus, intestinal hyperplasia in the nude mouse, CHO-IL-6 model of continuous IL-6 exposure is associated with prolonged enterocyte lifespan and decreased enterocyte migration rate, without increased proliferation.

**Effect of continuous recombinant murine IL-6 on intestinal enterocytes in mice**

To confirm that the observed increase in the number of enterocytes and villus length was specific to IL-6 and generalisable to...
immunocompetent mice, we implanted subcutaneous osmotic minipumps delivering recombinant murine IL-6 (1 ng/h) or carrier (water) into C57BL/6J mice. Seven days of IL-6 administration resulted in plasma IL-6 levels exceeding 4000 pg/ml at termination (figure 4A). Consistent with previous results, we observed a \( \approx 40\% \) increase in small bowel wet weight (figure 4B) as well as a \( \approx 52\% \) increase in villus length (figure 4D,F), again with no change in crypt depth (figure 4E). As in the CHO-IL-6 nude mouse model, PCNA levels were unchanged in Western blotting analysis of total jejunal lysates and expression of the enterocyte marker, villin, was unchanged (figure 4C).

**In vivo effects of IL-6 on pSTAT3 and apoptotic protein expression**

Based on the prolonged enterocyte lifespan observed with IL-6 treatment, we next sought to identify the pathways through which IL-6 might prevent enterocyte apoptosis. To define the enterocyte populations responding to IL-6, we performed immunohistochemistry for pSTAT3 on intestinal sections prepared after 24 h of IL-6 exposure. Nuclear localised pSTAT3 was observed in enterocytes outside the crypt, in the lower and middle sections of the villus following IL-6 exposure. Little-to-no pSTAT3 immunostaining was observed in enterocytes from control treated mice (figure 5A). Western blotting analysis of pooled total jejunum extracts was performed on mice sacrificed 1, 2, 3, 5 and 7 days following onset of IL-6 exposure. Similar Western blots were performed for the colon. Sustained induction of pSTAT3 was observed at all time points examined (figure 5B, C). Previously we observed that IL-6 induced phosphorylation of the anti-apoptotic protein Akt in the liver.\(^7\) \(^\text{15}\) Similarly, we observed dramatic induction of both total Akt and p-Akt levels in both small and large intestines from mice treated with IL-6. Akt
Figure 4  Recombinant murine IL-6 induced small intestinal hypertrophy in C57BL/6J mice. Recombinant murine IL-6 (200 ng) or carrier (water) was administered intraperitoneally by an Alzet pump over 7 days (1 ng/h). (A) Plasma IL-6 levels by ELISA at euthanasia (day 7). (B) Total small bowel wet weight as a fraction of total body weight. (C) Western blotting analysis of total jejunum lysates demonstrates no induction of PCNA following IL-6 exposure. (D) H&E staining of jejunum sections and (E) measurement of crypt depth and villus height in at least 15 well-oriented crypt/villus units from each of n=4 water treated or IL-6 treated mice. **p<0.001.

Figure 5  STAT3 activation in intestinal enterocytes from IL-6 treated mice and modulation of pro- and anti-apoptotic pathways. (A) Immunohistochemistry demonstrating nuclear pSTAT3 localisation in enterocytes of jejunum isolated from mice euthanised 24 h after injection of CHO-IL-6 or control cells. (B,C) Western blotting analysis of total jejunum extracts or colon isolated on the indicated day after initiation of IL-6 or control exposure. Each lane is equivalent amounts of tissue extract pooled from n=3 mice per point.

Activation is known to suppress apoptosis in normal intestinal epithelial cells by a variety of mechanisms including the inhibition of terminal caspases. Consistent with an inhibition of apoptotic pathways, Western blotting analysis showed reduced cleaved caspase-3 and 6 levels, as well as reduced levels of the Caspase-3 substrate, cleaved PARP. No change in caspase-9 levels was observed. In other cell types, Akt also functions to promote cell survival through phosphorylation of the Bcl-2 family member Bad. In this model, however, both total Bad and pBad were unchanged. Other studies have demonstrated potential anti-apoptotic activities for Akt including inhibition of the family of stress-activated kinases and IKKα activity that ultimately leads to NFκB activation and cell survival. These alternative pathways were not examined and remain additional candidate pathways mediating the anti-apoptotic effects of IL-6 through Akt.

In the liver IL-6 through STAT3 induces expression of additional anti-apoptotic proteins, most notably Bcl-2, Bcl-XL, ref-1 and FLIP. In the intestine, IL-6 exposure was associated with induction of the anti-apoptotic proteins FLIP and Ref-1, while Bcl-2 was down-regulated and Bcl-xL not much changed. Levels of FLIP in the small bowel were 1.6 times that of control intestines at days 3 and 5 following IL-6 exposure, and levels of Ref-1 were 1.8, 1.3 and 1.5 times that of control intestines at days 1, 3 and 5. Other anti-apoptotic proteins known to be regulated by IL-6 in the liver did not appear to change. Thus, IL-6 may exert its anti-apoptotic effects on the intestine through several cellular pathways including Akt phosphorylation and regulation of anti-apoptotic pathways.

Effects of IL-6 pretreatment on recovery from intestinal ischaemia reperfusion

Based on the in vivo anti-apoptotic effects of IL-6 on the normal small intestinal epithelium, we next examined the potential of exogenously administered IL-6 to ameliorate intestinal ischaemia–reperfusion injury. Mice were injected with CHO-IL-6 or control cells 4 days prior to surgery, when they were subjected to 30 min of ischaemia followed by 60 min reperfusion. Alternatively, mice were injected 3 days before surgery and subjected to 45 min of ischaemia and 60 min reperfusion. After 30 min of ischaemia and 60 min reperfusion, intestinal sections from control treated mice revealed severely damaged villi and separation and exfoliation of enterocytes. In IL-6 treated mice from the same group, much less intestinal injury was observed, with the injury confined largely to the villus tips (figure 6A). After 45 min of ischaemia and 60 min reperfusion, denuding extended to the...
midpoint of the villus or lower. In marked contrast, intestinal sections from IL-6 pretreated mice showed preservation of villus structure (figure 6A).

Immunohistochemistry for cCaspase-3 detects cells undergoing apoptosis. Intestinal sections from IL-6 treated mice demonstrated a substantially larger zone of cCaspase-3-negative cells from the bottom of the crypt to the region of positive staining at the villus tip, after either 30 or 45 min of ischaemia. Following 45 min of ischaemia, however, a greater number of cCaspase-3-positive cells were observed in IL-6 pretreated villus tips. Given the very short, denuded villi in control mice, this is likely due to shedding of dead and dying cells into the intestinal lumen, causing them to be lost in tissue processing and undetectable by immunohistochemistry.

TUNEL-positive cells were seen in approximately the top third of villi in intestinal sections from control treated mice after 30 min of ischaemia and 60 min reperfusion. Far fewer TUNEL-positive cells were seen in the IL-6 pretreated group, and these were generally limited to the villus tip with less shedding observed, consistent with the cCaspase-3 immunohistochemical staining (figure 6C). Quantitation of the TUNEL experiment demonstrated a 51% reduction in TUNEL-positive cells in the intestines of IL-6 treated mice (9.58 ± 2.15% vs 19.40 ± 4.97%, p < 0.01) (figure 6D). To assess intestinal barrier function following ischaemia—reperfusion injury, we examined the effects of IL-6 on intestinal permeability by assaying for plasma fluorescence of FITC-dextran given by gavage just prior to ischaemia. IL-6 pretreated mice demonstrated decreased serum FITC after 30 min of ischaemia—reperfusion injury versus controls, indicating that IL-6 treatment mitigated injury and improved intestinal barrier function (figure 6E). Of note, the serum concentrations in mice not subjected to intestinal ischaemia—reperfusion were less than 50 μg/ml. The Chiu score, a statistical measure of intestinal injury, was also calculated and demonstrated a dramatic preservation with IL-6 exposure (figure 6F).

IL-6 null mice have impaired recovery from intestinal injury
IL-6 is known to be induced following intestinal injury and is one of many ligands that bind to the gp130 receptor. Given the observation that IL-6 pretreatment resulted in decreased apoptosis, prolonged enterocyte lifespan, and lessened the degree of injury, we sought to determine whether endogenous IL-6 might play an essential, non-redundant role in intestinal recovery and repair. Wild-type colony-control mice and age-, sex- and strain-matched Il6−/− mice were subject to either of two intestinal injury models: 50% enterectomy or 5-FU administration. Il6−/− mice demonstrated significantly increased mortality over wild-type controls, with no Il6−/− mice surviving removal of 50% of the small bowel (figure 7A). In contrast, no difference in survival was observed between genotypes after simple intestinal transection and re-anastomosis. Thus the impaired survival of Il6−/− mice may not have been due to differences in early adaptation or altered host response to spillage of gut contents, but rather to altered gut injury and dysfunction after injury. Indeed, immunohistochemistry for cCaspase-3 in jejunum samples obtained 24 h after 50% enterectomy showed a greater than fourfold increased cCaspase-3 positive cells (predominantly...
Figure 7  Increased intestinal injury and death in Il6−/− mice reveals an essential, non-redundant role for endogenous IL-6. (A) Reduced survival in Il6−/− mice after 50% enterectomy but not after simple intestinal transection and re-anastomosis (n=6–8 per group, p<0.05). (B) cCaspase-3 staining in jejunum sections taken 12 h after 50% enterectomy shows increased cCaspase-3 positive cells in both the crypts and villus tips of Il6−/− mice. Quantitation of positive cells in the crypts of 15–30 well-oriented crypt/villus units were analysed for each of n=4 mice per group. **p<0.02. (C) Increased cCaspase-3 positive cells by immunohistochemistry in ileum and colon sections from 5-FU treated Il6−/− mice versus age- and sex- matched wild-type controls. No differences were observed in carrier treated mice. (D) Quantitation of cCaspase-3 positive cells in colon sections from 5-FU treated mice (15–30 well-oriented crypts per sample, n=4 per group). (E) Western blotting analysis of total colon or ileum extracts from 5-FU treated mice shows increased cCaspase-3 in Il6−/− mice. (F) Quantitation of (E) using Kodak1D 3.6, normalising to actin. *p<0.05; **p<0.02.
In the intestine, IL-6 is induced although the DSS suggesting a potential role for IL-6 in intestinal homeostasis.\textsuperscript{35} and increased mortality following cecal ligation and puncture, IL-six demonstrated increased cCaspase-3 in the ileum and colon in Il6\textsuperscript{−/−} mice versus controls (figure 7C−E). The enhanced enterocyte cell death and increased overall mortality in these two models of intestinal injury support the interpretation that endogenous IL-6 plays an important, non-redundant role in limiting intestinal injury and cell death.

**DISCUSSION**

IL-6 is a pleiotropic cytokine with a variety of effects on cells and tissues. IL-6 signals through the gp130 receptor, inducing phosphorylation of STAT3 but also activation of the mitogen activated protein kinase (MAPK) pathway. IL-6 activation of gp130 also requires a co-receptor in the form of membrane-bound gp80 or the corresponding soluble receptor generated by cleavage or alternative splicing.\textsuperscript{27} In the intestine, IL-6 is induced in several injury models including intestinal sepsis and after surgical intestinal manipulation.\textsuperscript{28,29} In vitro studies have shown that IL-6 prevents death of IEC-6 rat intestinal epithelial cells subjected to hypoxia.\textsuperscript{30,31} Conflicting in vivo reports have suggested both a detrimental and a protective role for IL-6 in recovery from intestinal ischaemia/reperfusion injury.\textsuperscript{32,33} IL-6 has been shown to preserve intestinal graft function, suggesting a protective effect in intestinal function; however, the exact mechanism of this protection remains unclear.\textsuperscript{34} IL-6 null mice have been shown to exhibit increased epithelial erosion following dextran sulfate sodium (DSS)-induced inflammatory colon injury and increased mortality following cecal ligation and puncture, suggesting a potential role for IL-6 in intestinal homeostasis.\textsuperscript{35}

In this study, we demonstrate that IL-6 is an important anti-apoptotic factor in intestinal homeostasis. Further, we demonstrate that sustained IL-6 exposure at intestinal concentrations observed in several in vivo intestinal injury scenarios resulted in a 30% to 50% increase in small bowel wet and dry weights with increased villus height. Sustained IL-6 administration resulted in down-regulation of several terminal apoptotic pathways in the intestine, likely through the apparent regulation of several pro- and anti-apoptotic proteins. Inhibition of the terminal cleaved caspases-3 and 6 as well as PARP was observed. Similar to some research relating to the liver, the effects of IL-6 appear to involve multiple pathways concerned with apoptosis and necrosis. IL-6 induces two important anti-apoptotic proteins, FLIP and ref-1, as well as phosphorylation of Akt (which directly inhibits terminal caspase activation in enterocytes). Conversely, evaluation of proliferative pathways by Western blot and intestinal labelling experiments shows no increase in proliferation rates in enterocytes in response to IL-6.

Given that our model primarily used nude mice that have a mutation in FOXN, a gene expressed in intestinal epithelium,\textsuperscript{36} we also investigated the effects of IL-6 delivered by an osmotic minipump in normal C57BL/6J mice. IL-6 administration did not increase intestinal PCNA expression. Thus, based on the data presented herein, the mechanism of increased intestinal mass observed following IL-6 exposure in vivo occurs via inhibition of apoptotic pathways that result in increased enterocyte longevity with no effect on proliferation rates. Figure 8 demonstrates the model for IL-6 anti-apoptotic activity on the gut based on these studies.

We also examined the effects of exogenous or endogenous IL-6 on various intestinal injury models. We hypothesised that IL-6 would prove protective and indeed, it dramatically increased intestinal survival, preserved villus architecture, and maintained mucosal barrier function. Of note, following lengthier periods of ischaemia, IL-6 also resulted in increased cCaspase-3 immunostaining in the villus tips. These results suggest that IL-6 may have resulted in preferential cell death via an apoptotic rather than a necrotic pathway, possibly due to improved energy stores in IL-6 treated enterocytes.

As IL-6 is not the only gp130 ligand induced after injury, we sought to define the non-redundant activities of IL-6 in mice. Previously, Jenkins and coworkers had noted increased mortality in Il6\textsuperscript{−/−} mice following DSS-induced colitis.\textsuperscript{37} Although the DSS injury is complex and involves a significant inflammatory response, we posited that a plausible mechanism for the increased mortality in Il6\textsuperscript{−/−} mice might be an increased sensitivity to apoptotic and necrotic stimuli. This is supported by the increased mortality and enterocyte cell death in the large and small intestines of mice subjected to 5-FU or in the remnant intestine after enterectomy. In the small intestine, increased apoptosis after 5-FU was observed in the crypts as well as the villi.

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**Figure 8** Model for IL-6 mediated inhibition of apoptosis in intestinal epithelial cells, with parallels to known anti-apoptotic pathways in hepatocytes. See text for details and references. Please compare diagram to Western blotting results in figure 5.
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Taken together, these data suggest that IL-6 plays a crucial regulatory role in vivo in enterocyte homeostasis, a function not dissimilar to the role played by IL-6 in the liver. IL-6 appears to affect enterocyte apoptotic pathways at several junctures, resulting in increased enterocyte resistance to extrinsic apoptosis, intrinsic apoptosis and oxidative injuries, thereby tipping the balance between life and death after injury. Investigation of the potential therapeutic application of IL-6 in intestinal disorders associated with increased enterocyte apoptosis such as extensive surgery for tumour extirpation or in intestinal transplant appears warranted.

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Competing interests None.

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