Interleukin 8 secretion by colonic crypt cells in vitro: response to injury suppressed by butyrate and enhanced in inflammatory bowel disease

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Abstract
Epithelia from several sites exhibit inducible secretion of interleukin 8 (IL-8). This study aimed to characterise secretion of IL-8 by colonic epithelial cells in vitro. Colonic crypt cells were isolated enzymatically from resected colon and the IL-8 content of culture supernates was measured by ELISA. The rate of secretion of IL-8 accelerated and levels of IL-8 transcripts increased appreciably during culture. Exposure to tumour necrosis factor α (TNFα) failed to increase secretion further. Secretion was not induced by the enzymatic digestion or by serum used in the culture medium but was significantly inhibited by butyrate, by a mean of 23%. Control experiments indicated that colonic crypt cells were the likely source. The secretion of IL-8 over 24 hours by cells from uninfamed mucosa of patients with ulcerative colitis or Crohn's disease was more than twofold that from normal cells, while that from cancer bearing colons was normal. TNFα (10 mM) significantly suppressed IL-8 secretion only in the ulcerative colitis group and the change was different to those in the normal (p=0.007) and Crohn's disease groups (p=0.012). Cells from inflamed areas secreted more IL-8 than those from autologous uninfamed areas (p=0.009) but responses to modulating factors were no different. The induction of IL-8 secretion by colonic crypt cells in vitro is probably a response to injury associated with isolation and culture. It is suppressed by butyrate and increased in inflammatory bowel disease independently of the presence of mucosal inflammation. Whether epithelial derived IL-8 plays a part in the pathogenesis of inflammatory bowel disease is not yet clear.

Keywords: colon, epithelium, interleukin 8, butyrate, inflammatory bowel disease, cell culture.

The colonic epithelium is the major barrier between the potentially injurious luminal environment and the internal milieu of the body. When this epithelium is breached, either because of epithelial injury or because of invasion by, for example, bacteria, polymorphonuclear leukocytes (PMN) migrate from the circulation up a chemotactic gradient to that injured area. Chemoattractant factors may originate from within the mucosa (for example, leukotriene B4 or interleukin 8 (IL-8)) or from the lumen (for example, bacterial derived peptides). Although the cellular origin of mucosal derived chemoattractants has long been thought to be mainly from inflammatory cells, recent studies have shown that, in vitro, epithelial cells, including keratinocytes,1 bronchial,2,3 and renal cortical cells4 in primary culture, and colon cancer cell lines5-7 are capable of releasing IL-8. This secretion seems to occur constitutively but is greatly enhanced when the cells are exposed to macrophage derived monokines such as tumour necrosis factor α (TNFα) or interleukin 1 (IL-1)1 2 4 5 7 or after infection of the cells by, for example, influenza virus A in bronchial epithelium,8 herpes simplex virus infection of human corneal keratinocytes,9 or invasive Salmonella species in a differentiated colon cancer cell line. Thus, the epithelium itself may be an important source of chemoattractants especially early in response to epithelial injury before other inflammatory cells are recruited to the injured area.

Normal human colonic epithelial cells have recently been shown to secrete IL-8 in vitro5 but the factors that might control this expression and whether there are disease differences are unknown. This study aimed to determine the pattern of secretion of IL-8 by isolated colonic crypt cells, to define some modulating factors, and to determine whether IL-8 release and its response to modulating factors differs according to the underlying large bowel disease.

Methods

PATIENTS AND SPECIMENS STUDIED
Details of patients from whom tissue was obtained are shown in Table 1. Macroscopically normal mucosa was obtained from large bowel surgically resected in 16 patients because of non-malignant large bowel disease. Seven patients had diverticular disease, two had constipation, one had rectal prolapse, one angiodysplasia, one colonic leiomyoma, three had recurrent sigmoid volvulus, and one patient with a normal large bowel had been misdiagnosed as having a caecal carcinoma by barium enema. The tissue from these patients was considered to represent normal large bowel mucosa.

In 20 patients, macroscopically normal mucosa was obtained from large bowel resected because of colorectal carcinoma. Tissue
was taken more than 10 cm from the edge of the tumour and adjacent mucosa was histologically normal. Duke's staging was A in four, B in six, and C in 10.

Mucosa was also obtained from 16 patients with ulcerative colitis. Adjacent mucosa was assessed histopathologically and judged to be uninfamed or inflamed on standard criteria. Using historical data and the current histopathology, tissue was obtained from seven apparently uninfamed, histologically normal segments, six previously inflamed now inactive segments, and nine actively inflamed segments (including five samples paired with apparently normal mucosa). Drug treatment comprised moderate to high dose corticosteroids in 14 (none in two), a mesalazine delivering drug in 11, azathioprine in one, and tinidazole in one.

Mucosa was also obtained from 14 patients with Crohn's disease. In 11 the mucosa was normal and in five it was inflamed (including one sample paired with an inflamed sample). Seven patients had Crohn's colitis, five ileocolitis, and two ileitis. Drug therapy in this group comprised moderate to high dose corticosteroids in 10 patients, azathioprine in two, a mesalazine delivering drug in six, and metronidazole or tinidazole in two. The procurement of colonic specimens was approved by The Royal Melbourne Hospital Board of Medical Research and Ethics Committee.

**ISOLATION OF COLONIC CRYPT CELLS**

Colonic crypt cells were isolated in the form of whole or partial crypts from resected mucosa using the collagenase/Dispase technique as previously described in detail. Cell populations were contaminated with less than 5% non-epithelial cells on morphological criteria and the viability of colonic epithelial cells immediately after isolation was always >98% as assessed by 0-1% trypan blue exclusion. The culture media in which the viability of crypt cell populations has been studied in detail previously comprised either Dulbecco's modification of Eagle's medium containing 20% Nu-serum-1 (Collaborative Research, Bedford, MA, USA), 25 mmol/l HEPES, 4 mmol/l L-glutamine, 50 mmol/l penicillin, 50 mg/ml gentamicin, and 100 mmol/l nystatin, or the same medium but without addition of Nu-serum. The criteria upon which the continuing viability of crypt cell populations that have been in culture for over 16–24 hours have been established and include rates of protein and DNA synthesis, and leakage of intracellular proteins from 51Cr-prelabelled cells.

**NORTHERN BLOT ANALYSIS**

Total RNA was extracted from both freshly isolated and cultured colonic crypt cell populations using the guanidium thiocyanate method. Total RNA (10 μg) was denatured with formamide and separated on 1-4% agarose-formaldehyde (2-2 mmol/l) gels. Ethidium bromide staining of the gel was used to assess the integrity of the mRNA in the population of the RNA. After overnight (18 hours) transfer of the RNA to a nylon membrane (Zeta-Probe, BioRad, Richmond, CA, USA), hybridisations were performed using a 1-3 kb complementary DNA probe for IL-8 (gift of Dr Karen O'Rourke), labelled with 32P-dATP (specific activity 106 cpm μg−1 μl−1) by random priming (Promega Corporation). Autoradiograms were exposed using two intensifying screens for up to two days at −70°C. For some experiments, membranes were stained for mRNA using a buffer containing 15 μmol/l sodium chloride, 1-5 μmol/l trisodium citrate, and 0-5% sodium dodecyl sulphate for 40 minutes at 95°C before probing.

**ASSAY OF IL-8**

Colonic crypt cells were cultured in 96 well U-bottomed plates in order to maintain the crypts in suspension. The culture medium described above was used with or without the addition of Nu-serum (final concentration 20%). For experiments examining modulation of IL-8 secretion, short chain fatty acids (SCFA), sodium butyrate, propionate, or acetate (all at pH 7-4), or TNFα (human recombinant; gift of Boeringer Mannheim), were added at the beginning of the culture period. SCFA were used at a concentration of 1 mmol/l since this concentration of butyrate achieves near maximal effects in colonic crypt cells and colon cancer cell lines for other biological parameters but is well below the limits of toxicity. TNFα was used only at a high concentration (10 nmol/l). The cells and medium were aspirated and centrifuged at 300 g for 5 minutes to separate the supernate from the cell pellet, which was then washed and mechanically homogenised in 1 ml of mannitol buffer (50 mmol/l mannitol and 2 mmol/l trizma base in dH2O, pH 7-4). Cell homogenates and supernatants were stored at −20°C until assayed. All studies were performed in triplicate or quadruplicate.

The IL-8 content of supernates was measured by ELISA (#RPN 2147, Amersham International, Little Chalfont, Buckinghamshire, UK). The results were expressed relative to the protein content of autologous cell homogenates; this was measured using bovine gamma globulin as standard.13 For experiments comparing the effect of serum on IL-8 secretion, levels were expressed relative to the DNA content of cell homogenates (measured using calf thymus DNA as standard18) because of previous observations that serum affects the protein content of colonic crypt cells.11 IL-8 was not detectable in Nu-serum. The coefficient of variation between replicate wells for all assays was <10%.
again with a cDNA to glyceraldehyde phosphate dehydrogenase (GAPDH), also random primed with 32P-dATP. Laser densitometry (Molecular Dynamics, Kew, Victoria) was used to compare the relative expression of IL-8 between lanes in individual experiments. Where RNA loading on gels was uneven, densities were normalised relative to that of GAPDH. RNA was run in duplicate or triplicate to ensure an acceptable coefficient of variance was achieved in densitometry measurements (<20%). The linear range of x-ray exposure was used in all experiments.

CONTROL EXPERIMENTS

Effects of the isolation process
The potential effect of the isolation process on the production of IL-8 by colonic epithelial cells was assessed in the colon cancer cell line, LIM1863 (gift of Dr Robert Whitehead, Ludwig Institute for Cancer Research, Melbourne). These cells grow in suspension in crypt-like structure17 and can be handled similarly to normal crypts. These were harvested by mild mechanical trauma and sieved through a fine stainless steel mesh to remove large cell lumps before the study. The cells were incubated for 1-5 hours in medium containing collagenase and Dispase, identical to that used in the isolation process, and then processed as for colonic crypts. A control population was treated similarly but without the enzymes in the medium. The production of IL-8 by the cells over the next 24 hours was then measured and compared.

Effects of contaminating non-epithelial cells
The non-epithelial cells contaminating colonic crypt populations have been previously characterised and comprise mostly mononuclear and plasma cells with some mesenchymal (vimentin-positive) cells.9 These cells were isolated from resected mucosa by first removing the colonic epithelium by incubation for 90 minutes in multiple changes of HBSS containing 3 mol/L EDTA followed by vigorous shaking. The mucosa devoid of epithelium (confirmed by histological examination of a sample of tissue) was scraped from the submucosa and subjected to collagenase/Dispase digestion, trituration, sieving, and washing steps as for colonic epithelial cells except that centrifugation was performed at 300 g for 5 minutes. The resulting population was contaminated with <5% epithelial cells, contained similar cell types to those contaminating crypt cell populations, and >95% were viable on trypan blue exclusion. The cells were then cultured and harvested in the same way as for colonic crypts and the IL-8 content of cell supernates measured.

Effect of TNFα on cell viability
Whether TNFα had any unspecific suppressive or toxic effect on colonic crypt cells was examined by determining its effect on the rate of protein synthesis over the last four hours of a 24 hour culture period. To do this, the specific uptake into acid-precipitated proteins of 14C- leucine was measured using methodology previously described in detail.11 12 The results were expressed as dpm per hour of exposure to 14C-leucine.

STATISTICAL EVALUATION
Data that approximate a normal distribution are expressed as mean (SEM) while non-parametric data are shown as median (range). For comparison of the effects of modulating factors between or across groups, the change in IL-8 concentration was expressed as a ratio of that with the modulating factor to that for the autologous control cells. All statistical analyses were performed using Microstat release 4.1 (EcoSoft Inc, Indianapolis, USA 1984). Paired/unpaired data were compared by paired Student’s t test or Wilcoxon rank sum tests respectively according to the number of replicate experiments performed and the distribution of data. Differences across groups were assessed by analysis of variance. The relationship between IL-8 secretion and age was evaluated by linear regression analysis and the Pearson correlation coefficient (r) calculated. Differences with a p value ≤0.05 were considered to be statistically significant.

Results

SECRETION OF IL-8 BY COLONIC CRYPT CELLS
There was a time dependent increase in the concentration of IL-8 in supernates of cultured colonic crypt cells and, as shown in Figure 1, the appearance of IL-8 seemed to accelerate in the period 12–18 hours compared with 0–6 hours. Secretion over 24 hours from the 60 crypt cell populations isolated from unflamed mucosa was 32 (4) ng/mg protein. The secretion of IL-8 was similar for cells cultured under serum-free conditions, in which the cells have been shown to remain viable on several criteria12 (15 (6) ng/µg DNA), as for autologous cells cultured with 20% Nu-serum (15 (5) ng/µg DNA; n=7). Age related or regional differences were not observed (data not shown).

Northern blot analysis showed that mRNA specific for IL-8 was always detectable in

![Figure 1: Pattern of secretion of interleukin-8 (IL-8) from colonic crypt cells over 18 hours' culture.](http://gut.bmj.com/)
freshly isolated colonic crypt cells (isolated from cancer bearing colons in all experiments). Its relative expression, however, increased appreciably during culture, as illustrated in Figure 2. In three separate experiments, IL-8 mRNA levels increased 30 to 100 fold following 4-6 hours' culture, and increased by an additional 64 to 120% following a further 14 hours' culture.

To compare levels of IL-8 secretion across disease groups, only results from cell populations isolated from uninfamed mucosa were used. A shown in Figure 3, levels of IL-8 differed significantly across the four disease groups (p=0.020). Secretion of IL-8 by cells from colon affected by ulcerative colitis and Crohn's disease was similar but in both cases was significantly greater than secretion in the normal group (p=0.007, 0.012, respectively). Secretion in the cancer group was not different from normal. The clinical (Dukes's) stage of the cancer did not influence the results (p=0.01; analysis of variance across the stages).

The effect of mucosal inflammation was examined in autologous pairs of cells from quiescent or apparently normal areas and from inflamed areas in five patients with ulcerative colitis and two with Crohn's disease. Secretion of IL-8 by cells from inflamed areas (median (range) 55 (4-287) ng/mg) was significantly greater than that from uninfamed areas (median (range) 10 (3-145) ng/mg; p=0.009, Wilcoxon signed rank test).

In patients with ulcerative colitis, the mucosa from which the colonic crypt cells were isolated was not simply classified according to the current state of inflammation (assessed histopathologically in the resected tissue). It was also classified in uninfamed mucosa according to whether inflammation had previously been present in that segment (determined by current histopathological features and findings from previous investigations including colonoscopic biopsies). Cells isolated from apparently unaffected mucosa secreted amounts of IL-8 (50 (20 ng/mg; n=7) similar to those obtained from mucosa previously affected but not currently inflamed (43 (14) ng/mg; n=6).

CONTROL EXPERIMENTS
LIM1863 cells processed identically to colonic crypt cells exhibited mild suppression rather than stimulation of IL-8 secretion (0.9 versus 0.5 ng/mg and 0.9 versus 0.6 ng/mg, respectively). Thus, the enzymatic digestion itself was unlikely to be stimulating IL-8 secretion.

The contribution made by the non-epithelial minority population contaminating the colonic crypt cell preparation to the secreted IL-8 was investigated by isolating lamina propria cells using an identical method to that for epithelial cells except that the epithelium was stripped from the mucosa using EDTA before the digestion. In four experiments (two in cancer bearing colons and two in uninfamed Crohn's disease), the lamina propria cells secreted 33, 23, 30, and 12 ng/mg protein respectively, which was similar to that secreted by autologous colonic crypt cells (17, 26, 30, and 25 ng/mg respectively). Since the colonic crypt cell populations usually comprise <5% non-epithelial cells, the contaminating cells contributed little to the total secreted IL-8.

EFFECT OF TNFα ON IL-8 SECRETION
To determine whether IL-8 secretion by colonic crypt cells could be further enhanced, the cells were exposed to TNFα at a relatively high concentration (10 nmol/l) for 24 hours. TNFα has been shown to stimulate potentially
IL-8 secretion in other cell types. At this concentration TNFα had no effect on the rate of protein synthesis after 20-24 hours’ exposure; in 13 colonic crypt cell populations (two normal, two cancer bearing, two Crohn’s disease, six ulcerative colitis), the uptake of 14C-leucine was 314 (64) dpm/h when exposed to TNFα compared with 320 (55) dpm/h in control cells. TNFα did not change the secretion of IL-8 from 30 colonic crypt cell populations isolated from histologically uninfammed mucosa (51 (7) ng/mg to 50 (7) ng/mg). Of individual disease groups, TNFα induced changes only in cells from ulcerative colitis where IL-8 secretion was suppressed by a small but statistically significant magnitude (p=0.037; Fig 4). The change induced by TNFα in the ulcerative colitis group (6 (3)% suppression; n=9) was significantly different from the change in the normal (25 (13)% stimulation; n=6; p=0.007) and Crohn’s disease groups (10 (6)% stimulation; n=8; p=0.012). The suppressive effect was not a result of unspecific suppression of protein synthesis since 14C-leucine uptake was similar in control (421 (94) dpm/h) and TNFα treated cells (443 (115) dpm/h) from six ulcerative colitis patients. There were no disease related differences in the response to TNFα in cells from inflamed or uninfammed mucosa in inflammatory bowel disease patients (data not shown).

EFFECTS OF SCFA ON IL-8 SECRETION

The effects of the physiologically relevant SCFA – butyrate, acetate, and propionate – at a concentration of 1 mmol/l on IL-8 secretion were evaluated in four colonic crypt cell populations isolated from cancer bearing colons. Twenty four hour exposure to butyrate caused a consistent and significant change in the IL-8 concentration in the supernate from 26 (6) ng/mg to 21 (5) ng/mg (p=0.012) while propionate (26 (7) ng/mg) and acetate (25 (7) ng/mg) had no effect. Butyrate at concentrations of 2 and 4 mmol/l also significantly (p<0.05) reduced secreted IL-8 in the autologous cells to 23 (5) ng/mg and 21 (4) ng/mg respectively, but a concentration dependent effect was not observed. In 56 cell populations from uninfammed mucosa examined, sodium butyrate (1 mmol/l) caused a modest suppression of IL-8 secretion after 24 hours by 23 (3)% from 33 (5) ng/mg to 26 (4) ng/mg (p<0.0001, paired t test).

The effects of butyrate on levels of mRNA for IL-8 were also examined in cell populations from cancer bearing colons and an example is shown in Figure 5. Northern analysis showed a 37% reduction in IL-8 mRNA levels in each of two separate experiments after 4 hours’ exposure to butyrate (1 mmol/l). A similar degree of
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Figure 6: The effects of butyrate (1 mmol/l) on secretion of interleukin 8 (IL-8) by colonic crypt cells according to the underlying disease. The results shown are expressed as percentage change of treated cells to control (untreated) cells. In every disease group, IL-8 secretion from butyrate treated cells was significantly less than that from control cells (p<0.05; paired t test). The numbers of patients studied in each group are shown in parentheses.

suppression of IL-8 mRNA was observed following exposure to butyrate for 18 hours (35 (7)%; n=4).

There were no differences in butyrate mediated changes across disease groups and, as illustrated in Figure 6, butyrate significantly suppressed IL-8 secretion in all patient groups. For patients with inflammatory bowel disease, butyrate mediated suppression was similar in presence (32 (8)%; n=11) or absence of mucosal inflammation (25 (4)%; n=23). No effects of age or regional differences were found on butyrate responses (data not shown).

Discussion

Colonic crypt cells from normal or diseased colons actively secrete immunoreactive IL-8 in vitro and express mRNA specific for IL-8. IL-8 found in culture supernatants was likely to be predominantly from colonic epithelium since lamina propria cells, a minority population (about 5%), secreted similar levels to crypt cells. The apparent acceleration of secretion during cell culture and the noticeable and rapid increase in mRNA for IL-8 indicated that the induction of IL-8 was occurring in vitro. The enzymatic digestion itself was unlikely to be the stimulus since similar exposure of LIM1863 cells to the enzymes did not increase their level of secretion. Likewise, IL-8 secretion did not result from stimulation by factors contained within serum: cells cultured in medium containing nutrients and buffer the secreted amounts of IL-8 similar to those cultured with serum. It is more likely that the stimulus was the cell ‘injury’ associated with the disaggregation process and culture in vitro. These data support the view that induction of IL-8 is an early epithelial response to injury.

Whether the colonic crypt cells could be further stimulated to secrete IL-8 was addressed by exposing the cells to a high but non-toxic concentration of TNFα, a cytokine that potently induces IL-8 production in many other cell types including colon cancer cell lines. No consistent effect was observed suggesting that the colonic epithelial cells approach maximal stimulation during their isolation and culture. Unexpectedly, exposure of colonic crypt cells from patients with ulcerative colitis to TNFα resulted in significant suppression of IL-8 secretion that was not attributable to unspecific suppression or toxicity of the cells. This change was significantly different from that seen with both normal cells and those from patients with Crohn’s disease. Though the small magnitude of the effect is of little likely relevance in vivo, it does demonstrate an ulcerative colitis specific abnormality in responsiveness of colonic epithelial cells. The finding was also independent of the presence of inflammation suggesting that the abnormality in the control of IL-8 secretion may be a primary rather than a secondary phenomenon in that disease. However, more detailed analysis of the time course and concentration effect of the inhibition are required before such a conclusion can be definitively reached.

Colonic crypt cells from histologically uninfamed mucosa from patients with either ulcerative colitis or Crohn’s disease exhibited significantly enhanced secretion over 24 hours compared with essentially untreated (or cancer bearing) large bowel. Secretion was even higher in cells obtained from inflamed mucosa. Of other potential variables, drug therapy is one that it is impossible to control in a study of this type since none of the patients in the normal or cancer groups were taking anti-inflammatory or immunosuppressive therapy, but there were no age related or regional differences in IL-8 secretion. The proportion of non-epithelial cells in the crypt cell populations isolated from mucosa of patients with inflammatory bowel disease is similar to that seen in non-inflammatory conditions but the relative proportion of IL-8-secreting subtypes, such as macrophages, may be different. To attribute the twofold increase in IL-8 secretion seen in the uninfamed inflammatory bowel disease group to non-epithelial cells, however, implies a massive output of IL-8 from a very small subpopulation of cells. Furthermore, IL-8 secretion from lamina propria cells under the same conditions was similar in magnitude to that from crypt cell populations isolated from uninfamed inflammatory bowel disease mucosa. A significant contribution of non-epithelial cells to the increased levels secreted by cells from inflamed mucosa cannot, however, be excluded.

Two pathogenic explanations for the enhanced IL-8 secretion in vitro from colonic crypt cells in inflammatory bowel disease are proposed. Firstly, the cells may be activated before isolation (that is, in vivo). This might occur if mucosal inflammation were present, especially since the mucosal IL-8 content is noticeably high where there is active inflammation. Although the crypt cells were obtained from mucosa that was judged to be normal or uninfamed on histopathological criteria, mild degrees of inflammation may have been present in some specimens. Furthermore, raised IL-8 concentrations have been reported in inactive mucosa in patients with ulcerative colitis. Whether this also
applies to mucosa from apparently unaffected areas is unknown but crypt cells from unaffected regions also demonstrated raised IL-8 secretion in the present study. Preliminary reports of in situ expression of IL-8 transcripts by colonic epithelium have shown positive evidence for its transcription in vivo only when reverse transcription polymerase chain reaction amplification was used.22-24 The second possibility is that the cells are exhibiting increased responsiveness to injury. Excessive secretion of IL-8 in response to a given stimulus might lead to a degree of inflammation that is inappropriate to that stimulus and, therefore, may be of primary pathogenic importance. In situ studies of colonic epithelium in actively inflamed mucosa22 23 have indicated that the epithelium is not a major producer of IL-8 in advanced lesions in patients with inflammatory bowel disease. This finding does not, however, negate the possibility that epithelial derived IL-8 is involved in the genesis of early lesions. These issues need to be addressed further.

Increasing evidence points to a crucial role for SCFA in the maintenance of the health of colonic epithelium. Butyrate, the principal substrate for the colon, is oxidized by the colon to appreciably reduced concentrations of luminal SCFA by, for example, diverting the faecal stream, leads to epithelial and mucosal atrophy25-27 and, in humans, mucosal inflammation (diversion colitis).28 Butyrate seems to be the most efficient SCFA in correcting these abnormalities.27 29 The basis of this apparent cause-effect relationship probably lies in the importance of butyrate as the major energy substrate for colonic epithelial cells especially in distal colon and rectum.30 The efficacy of butyrate enemas in distal ulcerative colitis31 is, however, less well explained by a butyrate mediated repulsion of the energy state of the epithelium since the actual oxidation of butyrate is impaired in epithelium associated with ulcerative colitis.32 It seems likely then that butyrate acts via additional mechanisms. It is well documented that butyrate modules appreciably the proliferation and differentiation of many cancer cell lines33 but these effects cannot be reproduced in colonic crypt cells in vitro.11 The findings in the present study are the first to show that butyrate can suppress the production and release of a primary inflammatory cytokine, IL-8. A pathogenic role for IL-8 in ulcerative colitis seems likely because mucosal levels of IL-8 are noticeably increased in inflamed mucosa and the prominent place that PMNs have in the histopathology of the mucosal lesion, even in mildly active disease, gives biological feasibility to release of IL-8 early in the pathogenesis of mucosal inflammation. An epithelial origin for the raised mucosal IL-8 concentrations, however, has yet to be supported.22 23 IL-8 is not the only potentially pro-inflammatory molecule suppressed by butyrate. The release from colonic epithelium of the neutral protease, urokinase, which is found in increased concentrations in the mucosa of patients with active ulcerative colitis,34 35 is also suppressed by butyrate in vitro,36 and indirect evidence in rats suggests such an effect of butyrate may also occur in vivo.37

Whether the modest suppression of IL-8 secretion in vitro by butyrate has any biological relevance in vivo has not been addressed in the present study. The likelihood that IL-8 synthesis and secretion by colonic crypt cells in vitro are induced to near maximal levels indicates that the injury associated with isolation and culture is a potent stimulus. A mean inhibition of 22% exerted by butyrate may, in the presence of such a stimulus, be of considerable biological significance in vivo where the stimuli to its secretion may be less potent.

In conclusion, the present study shows that normal colonic crypt cells are capable of secreting IL-8 in vitro and the processes of cell isolation and culture seem to induce its transcription, synthesis, and secretion. TNFα did not further stimulate its secretion suggesting near maximal levels of induction of IL-8 were occurring. Sodium butyrate inhibits the secretion of IL-8 and reduces mRNA levels. Colonic crypt cells from uninfamed mucosa of patients with ulcerative colitis or Crohn’s disease exhibit enhanced secretion of IL-8 in vitro. IL-8 gene expression in human bronchial epithelial cells38 shows that the IL-8 is released by colonic epithelium early in response to injury and suggest that the cells have heightened responsiveness in patients with inflammatory bowel disease. Whether epithelial derived IL-8 is an important pathogenic factor in inflammatory bowel disease, however, is still unexplained.

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