

Interleukin 2 modulates ion secretion and cell proliferation in cultured human small intestinal enterocytes

E V O'Loughlin, G P Pang, R Noltorp, C Koina, R Batey, R Clancy

Abstract

Aims—To determine if interleukin 2 (IL-2) alters epithelial transport and barrier function in cultured human small intestinal enterocytes.

Methods—Confluent monolayers of small intestinal cells derived from duodenal biopsies were treated with IL-2 0.2–50 U/ml for 24 hours prior to study. Transport measurements were performed under short circuited conditions in Ussing chambers, with and without the secretagogues forskolin and 3-isobutyl-1-methyl xanthine (IBMX). Serosal to mucosal flux of ³[H] mannitol (permeability) and ³[H] thymidine uptake (proliferation) were measured. IL-2 receptor and cystic fibrosis transmembrane conductance regulator (CFTR) mRNA were identified using reverse transcription-polymerase chain reaction (RT-PCR).

Results—IL-2 did not alter baseline electrical parameters but caused a significant increase in cAMP dependent chloride secretion. The effect was mediated by the IL-2 receptor and paralleled a rapid increase in tyrosine phosphorylation, janus kinase 1, and signal transducers and activators of transcription (STATs) 1, 3, and 5. IL-2 significantly increased proliferation but at a lower dose than observed for enhanced secretion but did not alter permeability. IL-2 receptor β and γ chains and CFTR mRNA were identified by RT-PCR.

Conclusions—IL-2 treatment enhances cAMP stimulated chloride secretion and cellular proliferation in a human small intestinal cell line expressing a functional IL-2 receptor.

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Keywords: interleukin 2; ion secretion; cell proliferation; enterocytes; small intestine

The ability to regulate epithelial fluid and electrolyte secretion provides a potent defence mechanism to protect the mammalian gastrointestinal tract against pathogenic organisms, noxious dietary antigen, and parasitic infestation. Secretion of fluid and electrolytes, while contributing to diarrhoea, enables the host to flush the lumen in an attempt to expel potential invaders. In recent years, it has become apparent that the mucosal immune system plays an

important role in regulating fluid and electrolyte secretion as an integrated component of mucosal host defence.¹

Several mucosal immunocytes have the ability to regulate epithelial transport including mast cells,^{2–6} polymorphonuclear neutrophils,^{7–10} and peripheral blood monocytes¹¹ by releasing a range of secretagogues such as eicosanoids, histamine, serotonin, and 5'AMP. Lymphocytes are also important mediators of inflammation in the gastrointestinal tract but little is known about their role in modulating epithelial function. Isolated intraepithelial lymphocytes from mice intestine stimulate enterocyte proliferation when cocultured with small intestinal enterocytes.¹² Moreover, activation of mucosal lymphocytes in the lamina propria of fetal small intestine grown in organ culture stimulates crypt proliferation and villus atrophy.¹³ Few studies have addressed the question of whether lymphocytes modulate epithelial transport. McKay and colleagues¹¹ cocultured T84 cells with circulating human lymphocytes but found that the activated lymphocytes did not alter ion transport or barrier function in epithelial cells. However, given their role in many inflammatory disorders of the intestine, it is conceivable that lymphocytes could modulate epithelial transport of fluid and electrolytes.

Interleukin 2 (IL-2) is an important cytokine secreted by lymphocytes and plays a major role in stimulating proliferation of mucosal lymphocytes, natural killer cells, and macrophages. Until recently, its role was thought to be restricted to modulating the growth of immunocytes. However, functional IL-2 receptors have been identified in several intestinal cell culture lines, in rat small intestinal enterocytes, and in human colonocytes,^{14 15} implying a physiological role for IL-2 in modulating epithelial function.

We have previously described cultured human small intestinal cell lines derived from endoscopic biopsies.¹⁶ One such line, BN, grows in a polarised monolayer, morphologically resembles immature small intestinal crypt

Abbreviations used in this paper: CFTR, cystic fibrosis transmembrane conductance regulator; G, conductance; IBMX, 3-isobutyl-1-methyl xanthine; IL, interleukin; IL-2R, interleukin 2 receptor; DMEM, Dulbecco's modified Eagle's medium; Isc, short circuit current; JAK, janus kinases; PD, potential difference; J, flux; STAT, signal transducers and activators of transcription; PBS, phosphate buffered saline; RT-PCR, reverse transcription-polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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cells with a rudimentary brush border expressing low levels of sucrase, and exhibits vectorial transport. When studied under voltage clamp conditions in Ussing chambers, monolayer resistance varies from 30 to 200 Ω and addition of secretagogue to the bathing solutions stimulates an increase in apical chloride secretion. Preliminary studies coincubating the monolayers with isolated tonsillar lymphocytes resulted in an increase in secretagogue stimulated secretion (unpublished observations). We postulated that factors such as IL-2 required to propagate the lymphocytes may have been responsible for this effect.

The aims of this study were to examine further the effect of IL-2 on epithelial function. Specifically, we studied the effect of IL-2 on ion secretion, cellular proliferation, and epithelial permeability utilising a human small intestinal cell culture line, BN¹⁶.

Methods

CELL CULTURES AND IL-2 TREATMENT

Clones of human small intestinal enterocytes (BN) were obtained from a single patient as previously described.¹⁶ Cells were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, L-glutamine, and antibiotics. Cells were split after treatment with trypsin-versene and cultured on permeable supports (Falcon cell culture inserts, 4 μm pore and 24.5 mm diameter; Becton Dickinson, Franklin Lakes, New Jersey, USA) in a six well culture plate for 48 hours. After reaching confluence, the medium was removed from the monolayers and replaced with fresh medium containing rIL-2 at doses ranging from 0.2 to 50 U/ml and in some experiments rIL-15 0.5–5 ng/ml (doses in U or ng as per the manufacturers' instructions). Each experiment was performed with paired control monolayers which contained medium alone. The cells were grown for a further 24 hours at which time confluence of the monolayers was verified under an inverted microscope and monolayers were then studied.

IN VITRO TRANSPORT STUDIES

Confluent monolayers were mounted in Ussing chambers in quadruplicate and studied as previously described.¹⁶ Each chamber exposed 1.12 cm^2 surface area to apical and basolateral reservoirs containing 10 ml of Krebs buffer at 37°C, pH 7.4, containing (mmol/l): Na^+ 140; Cl^- 127.5; HCO_3^- 25; K^+ 10; Ca^{2+} 1.25; Mg^{2+} 1.1; H_2PO_4^- 2 with mannitol 5 mmol on the mucosal side and glucose 5 mmol on the serosal side. Buffers were oxygenated with 95% O_2 and 5% CO_2 and the buffers circulated by gas lift. Spontaneous transepithelial potential difference (PD, millivolts (mV)) was determined and the monolayer clamped at zero voltage by introducing a continuous short circuit current (Isc microamperes (μAmp)) by automatic voltage clamp (DVC 1000; World Precision Instruments, New Haven, Connecticut, USA). Open circuit PD was measured for 3–5 seconds every 10 minutes and conductance (G, milli-Siemens (mS)) determined by voltage clamping the monolayer at 10 mV and measuring the

resultant Isc. G was calculated from ΔPD and ΔIsc using Ohm's law. Monolayers were discarded when G was $>15 \text{ mS}/\text{cm}^2$. Isc was continuously recorded by pen chart recorder and electrical parameters recorded at 10 minute intervals after an initial 10 minute baseline equilibration period.

Secretagogue stimulated Isc

Electrical parameters were measured during a basal period followed by a second period when the secretagogues forskolin 10^{-5} M and IBMX (3-isobutyl-1-methyl xanthine) $3 \times 10^{-4} \text{ M}$ were added together to both sides of the monolayers.¹⁷ Change in Isc (ΔIsc) was determined from the peak increase after secretagogue addition. Time course experiments were performed to determine the time of onset of action of IL-2. Cells were incubated with IL-2 5 U/ml for 30 minutes, two hours, and 24 hours. Initial experiments indicated that 24 hour incubation of the cell culture with 5 U/ml IL-2 induced the maximum increase in secretagogue stimulated Isc. This time point was used in subsequent experiments. Experiments were repeated in chloride free buffer in which chloride salts were substituted with gluconate salts.¹⁸ To determine if IL-2 enhancement of secretagogue stimulated secretion was mediated via the IL-2 receptor, cells were incubated with anti-CD122 (anti-IL-2R β chain antibody; Pharmingen, San Diego, California, USA) 0.5 $\mu\text{g}/\text{ml}$, added six hours after seeding the cells. IL-2 5 U/ml was added at 24 hours and studies performed at 48 hours post seeding. Treatment of monolayers with a mouse IgG2, anti-IL-4 β chain antibody (an irrelevant antibody of similar isotype) had no effect on baseline electrical parameters or secretagogue stimulated Isc.

The specificity of the IL-2 effect on secretagogue induced secretion was examined in experiments where cells were treated with IL-15 (0.5–5 ng/ml) using the same protocol as for IL-2. IL-15 receptor shares common β and γ chains with IL-2R and induces similar biological effects.¹⁹

Epithelial permeability

Monolayer permeability was assessed by measuring serosal to mucosal flux of ^3H mannitol. Isotope (10 μCi) was added to the basolateral side and allowed to equilibrate for 10 minutes. Flux measurements were determined using standard techniques as previously described²⁰ during three 10 minute and one overall 30 minute period. Serosal to mucosal flux (J_{s-m}) was calculated and data are expressed as $\mu\text{mol}/\text{cm}^2/\text{h}$.

THYMIDINE INCORPORATION INTO BN CELLS

BN cells from monolayer cultures were seeded in 100 μl aliquots at 5000–10 000 cells/well in DMEM/10% fetal calf serum into wells of a 96 well flat bottomed microtitre plate. Medium alone (100 μl) or medium containing IL-2 (0.2–40 U/ml) was added in triplicate into the wells. After 24 hours of incubation, the cultures were pulsed with ^3H thymidine, 1 $\mu\text{Ci}/\text{well}$ for the final six hours before harvesting and counting in a liquid scintillation counter.²¹ Results

Table 1 Polymerase chain reaction primer pairs used for detection of α , β , and γ chains of interleukin 2 receptor (IL-2R), cystic fibrosis transmembrane conductance regulator (CFTR), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes

| | |
|----------------|--|
| IL-2R α | |
| Sense | 5' TCTTCCATCCACATCCTC |
| Antisense | 3' TCTGGGAAACCTCTCTTGC |
| IL-2R β | |
| Sense | 5' GGCTTTTGGCTTCATCATCT |
| Antisense | 3' CTTGTCCCTCTCCAGCACTT |
| IL-2R γ | |
| Sense | 5' ACGGGAACCCAGGAGACAGG |
| Antisense | 3' AGCGGCTCCGAACACGAAAC |
| GAPDH | |
| Sense | 5' ACCACAGTCCATGCCATCAC |
| Antisense | 3' ATGTCGTTGTCCACCACCT |
| CFTR | |
| Sense | 5' CAGAACTGAACTGACTCGG (exon 7-5') |
| Antisense | 3' GTTGGCATGCTTTGATGACGCTTC (exon 10-3') |

Table 2 Electrical parameters in control and interleukin 2 (IL-2) treated BN monolayers

| | PD (mV) | Isc (μ Amp/cm ²) | G (mS/cm ²) |
|---------------|--------------|-----------------------------------|-------------------------|
| Control | -0.29 (0.06) | 0.59 (0.17) | 4.29 (0.35) |
| IL-2 (5 U/ml) | -0.16 (0.05) | 0.79 (0.20) | 4.38 (0.32) |
| p | NS | NS | NS |

Values are mean (SEM) of 32 paired monolayers. Means were compared using the paired Student's *t* test.

PD, potential difference; Isc, short circuit current; G, conductance.

are reported as per cent of non-IL-2 treated controls.

RT-PCR DETECTION OF IL-2, IL-15 RECEPTORS, AND CFTR mRNA

Complementary DNA (cDNA) synthesis and amplification using reverse transcription-polymerase chain reaction (RT-PCR) have been previously described.¹⁶ The PCR primer pairs for detection of α , β , and γ chains of the IL-2 receptor, cystic fibrosis transmembrane conductance regulator (CFTR), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes, used according to published sequences, are shown in table 1.^{15 22 23}

IMMUNOBLOT DETECTION OF TYROSINE

PHOSPHORYLATED AND JAK AND STAT PROTEINS

BN cells were grown in transwells with six well cluster plates (Becton Dickinson) for 36 hours in DMEM containing 10% fetal calf serum. The cells were then washed in phosphate buffered saline (PBS) and recultured in serum free medium with 5 U of IL-2 for various times. At each time point, 300 μ l of cell lysis buffer (250 mM Tris HCl, pH 6.8, 4% sodium dodecyl sulphate, 10% glycerol, 2% 2-mercaptoethanol, 1 mM phenylmethylsulphonyl fluoride) heated at 95°C for five minutes was added to the insert. The lysate was collected, heated for two minutes, and centrifuged at 5000 rpm for five minutes in a Microfuge. Samples were resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred to Immobilon membranes (Millipore, North Ryde, NSW, Australia). Membrane blots were blocked with 5% non-fat skim milk at 4°C overnight before incubating sequentially with anti-janus kinase 1 (JAK1) (1:1500), anti-signal transducers and activators of transcription (STAT)-1, STAT-3 (1:1000), and STAT-5 (1:250), or antiphosphotyrosine (1:1000) mouse monoclonal antibodies (Santa Cruz Biotechnology, California, USA), biotinylated sheep antimouse IgG (1:1000), and horseradish peroxidase conjugated streptavidin (1:2000) (Amrad Biotech, Melbourne, Australia) according to the manufacturer's instructions. Signals were detected by enhanced chemiluminescence (ECL, Amersham, Piscataway, USA).

ANALYSIS OF DATA

Values are depicted as mean (SEM). Because of the variability of the Isc response to secretagogue in different preparations of BN cells, the secretagogue stimulated Isc is depicted as per cent increase over paired non-treated control

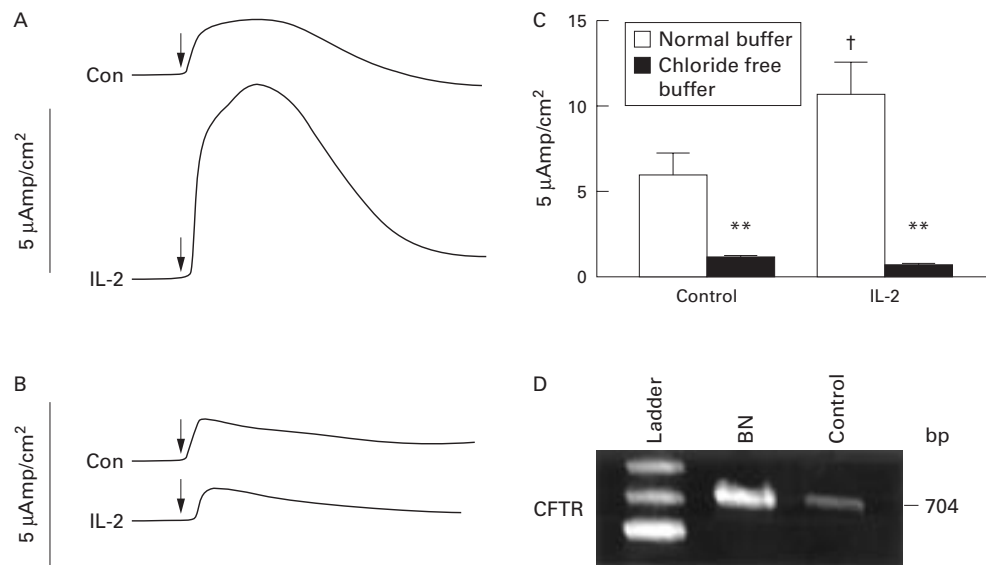


Figure 1 Effect of interleukin 2 (IL-2) on cAMP dependent chloride secretion in BN cells. (A) Representative traces of control (Con) and IL-2 treated monolayers. (B) Representative traces of control and IL-2 treated monolayers in chloride free buffer. (C) Secretagogue stimulated short circuit current (Isc) in normal buffer and paired monolayers in chloride free buffer ($n=7-9$ pairs, data are mean (SEM)). (D) Constitutive expression of cystic fibrosis transmembrane conductance regulator (CFTR) mRNA in BN cells and controls (human small intestinal biopsy). Cells depicted in (A-C) were treated with IL-2 5 U/ml for 24 hours prior to the study; secretion, stimulated with 3-isobutyl-1-methyl xanthine (IBMX) 3×10^{-4} M and forskolin 10^{-6} M (arrows). bp, base pairs. ** $p < 0.01$ compared with control and † $p < 0.02$ compared with respective control period by paired *t* test.

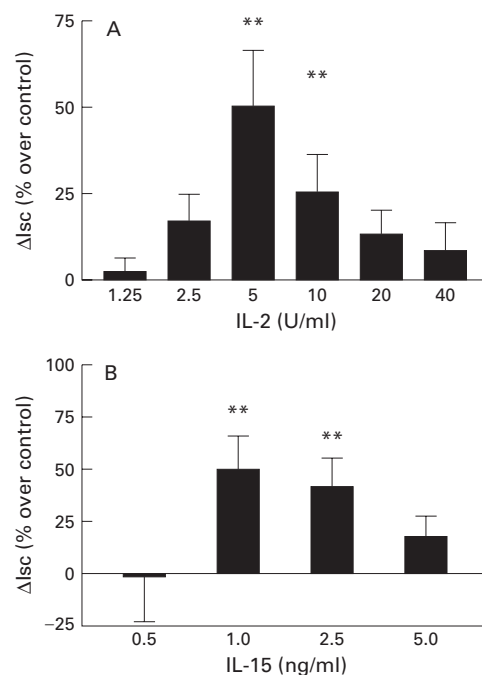


Figure 2 Dose response of (A) interleukin 2 (IL-2) and (B) interleukin 15 (IL-15) secretagogue stimulated short circuit current (Isc). Cells were incubated for 24 hours with either IL-2 or IL-15. Anion secretion was stimulated with forskolin 10^{-5} M and 3-isobutyl-1-methyl xanthine (IBMX) 3×10^{-4} M. Data are mean (SEM). Change in Isc (Δ Isc) is depicted as percentage of paired control monolayer, $n=14-31$ pairs. ** $p < 0.01$ compared with control by paired t test.

values unless otherwise stated. The effect of IL-2 on study parameters was assessed by the paired Student's t test with Bonferroni correction for multiple analyses. The effect of different doses of IL-2 on 3 [H] thymidine uptake was assessed using analysis of variance. If differences were significant, means were compared using Dunnett's test for multiple comparisons. $p < 0.05$ was considered significant.

Results

CHLORIDE SECRETION

IL-2 had no obvious effect on the ability of BN cells to form a confluent monolayer. Baseline electrical parameters are depicted in table 2 for monolayers treated with 5 U/ml IL-2, the dose which caused greatest enhancement of secretagogue stimulated Isc. Consistent with our previous observations, untreated BN cells exhibited low PD and Isc compared with other intestinal cells such as T84 cells.^{24, 25} Twenty four hour treatment with IL-2 caused no change in any baseline electrical parameter. Thus IL-2 treatment had no effect on baseline electrogenic ion transport properties of the cell monolayers.

IL-2 treatment at low doses enhanced the effect of the secretagogues IBMX and forskolin in stimulating Isc. Data are depicted in figs 1 and 2. BN cells treated with 5 and 10 U/ml IL-2 exhibited a significant increase in Isc ($p < 0.001$ compared with paired controls). There was slight enhancement of Isc at the lower dose of 2.5 U/ml and at doses greater than 10 U/ml but these changes were not

statistically significant. Subsequent experiments examining chloride secretion utilised 5 U/ml IL-2 treatment.

To determine whether enhancement of secretagogue stimulated secretion was limited to IL-2, experiments were repeated utilising IL-15 and an unrelated interleukin IL-4. IL-15 exhibited a dose dependent enhancement of secretagogue stimulated secretion (fig 2B) in contrast with IL-4 which had no effect (data not shown).

We have previously demonstrated that forskolin stimulated Isc in BN cells is due largely to chloride secretion as it is inhibited by chloride free buffer and apical addition of the chloride channel blocker diphenylamine-2-decarboxylate.¹⁶ To confirm that IL-2 treatment enhanced chloride secretion in response to forskolin/IBMX, experiments were repeated in chloride free buffer (fig 1C). In this group of experiments, IL-2 significantly increased secretagogue stimulated Isc from 5.96 (1.26) to 10.83 (1.88) μ Amp/cm² ($n=7$ pairs; $p < 0.02$). Removing chloride from the buffer caused a significant reduction in secretagogue stimulated Isc in both control and IL-2 treated monolayers ($p < 0.01$), and inhibited any IL-2 induced enhancement. The data suggest that IL-2 enhances cAMP dependent chloride secretion. This pathway of secretion is mediated by CFTR in the gastrointestinal tract.²⁵ Indeed, CFTR mRNA was detected in these cells by RT-PCR (fig 1D) although IL-2 treatment was not found to increase CFTR mRNA at 24 hours. Forskolin/IBMX stimulated Isc (Δ Isc) not inhibited by chloride free buffers constituted approximately 20–25% of the total stimulated Isc. This chloride independent Δ Isc was significantly reduced by IL-2 treatment: 0.83 (0.09) μ Amp/cm² in IL-2 treated monolayers *v* 1.23 (0.10) in controls; $p < 0.02$, $n=13$ pairs) (see fig 1C).

The time course of the IL-2 effect on secretion was examined in a separate group of monolayers and the data are depicted in fig 3. Thirty minutes of exposure of the monolayers to IL-2 induced a slight but non-significant increase in secretagogue stimulated Isc. However, by two hours this had increased further to 36 (6)% ($p < 0.01$) and remained elevated at 24 hours. In

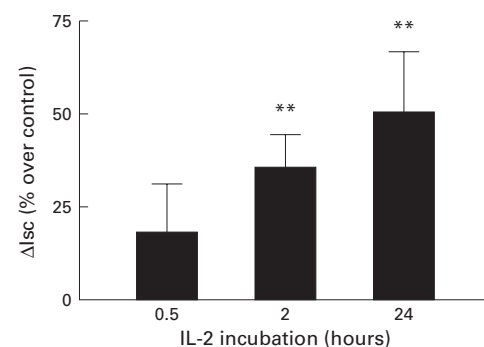


Figure 3 Time course of interleukin 2 (IL-2) effect on secretagogue stimulated short circuit current (Isc). Cells were treated with IL-2 5 U/ml. Anion secretion was stimulated with forskolin 10^{-5} M and 3-isobutyl-1-methyl xanthine (IBMX) 3×10^{-4} M. ** $p < 0.01$ compared with paired control monolayers. $n=7, 14,$ and 23 pairs at 0.5, 2, and 24 hours, respectively.

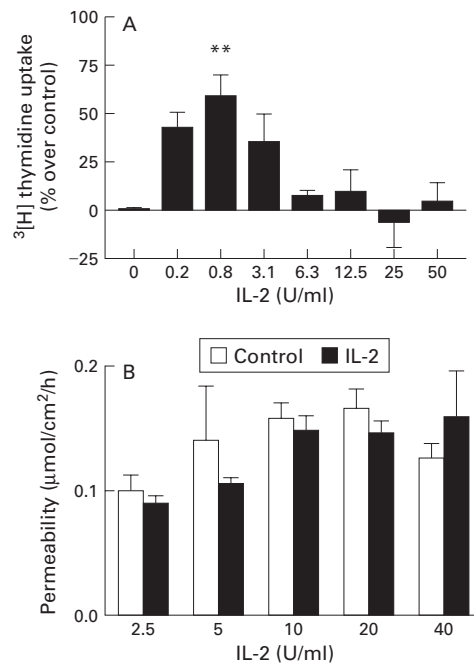


Figure 4 Dose-response of interleukin 2 (IL-2) effect on (A) cellular proliferation and (B) mannitol permeability. In (A), $n=3$ dose-response experiments. $**p<0.01$ compared with untreated monolayers. In (B), mannitol fluxes are serosal to mucosal in $n=13-23$ pairs.

contrast, IL-2 did not cause any significant alteration of electrical conductance at any time point.

The enhanced effect of IL-2 incubation on secretagogue stimulated Isc was inhibited in four of five monolayers pretreated with the IL-2 receptor antibody anti-CD122. Control monolayers, without IL-2 treatment, exhibited an increase in Isc of 6.3 (0.4) $\mu\text{Amp}/\text{cm}^2$ which was not significantly different to IL-2 treated (5

U/ml, 24 hours) monolayers which had been pretreated with anti-CD122 antibody (5.6 (1.6) $\mu\text{Amp}/\text{cm}^2$).

CELL PROLIFERATION

These studies were performed without prior depletion of fetal calf serum from the culture medium. Despite this, IL-2 treatment stimulated cell proliferation at low doses. Treatment with 0.2–3.1 U/ml caused an increase in ³[H] thymidine incorporation after 18 hours of incubation. However, this increase was only significant at a dose of 0.8 U/ml (159 (11.3)%; $p=0.001$ compared with control) (fig 4A).

MANNITOL PERMEABILITY

Preliminary experiments with BN cells assessed unidirectional and net fluxes of ³[H] mannitol. Mucosal to serosal flux (J_{ms} , 0.165 (0.07) $\mu\text{mol}/\text{cm}^2/\text{h}$; $n=13$) was not significantly different to serosal to mucosal flux (J_{sm} , 0.141 (0.06) $\mu\text{mol}/\text{cm}^2/\text{h}$; $n=13$). J_{sm} was used in subsequent measurements of monolayer permeability. IL-2 treatment had no significant effect at any dose between 2.5 and 40 U/ml (fig 4B)

IL-2 AND IL-15 RECEPTOR mRNA

Transcripts for α , β , and γ chains were identified by northern blot analysis in peripheral blood mononuclear cells, as expected (fig 5, control lane). However, BN cells expressed transcripts for β and γ but not α chains. This was compared with T84 cells, in lane 3, in which only γ transcripts were expressed.¹⁵ IL-15 α chain mRNA was not detected (not shown).

TYROSINE PHOSPHORYLATION AND STAT PROTEIN EXPRESSION

IL-2 stimulated tyrosine phosphorylation of multiple protein bands in BN cells (fig 6) as previously reported for intestinal epithelial cells.¹⁵ In addition, there was a time dependent increase in expression of JAK1, STAT-1, -3,

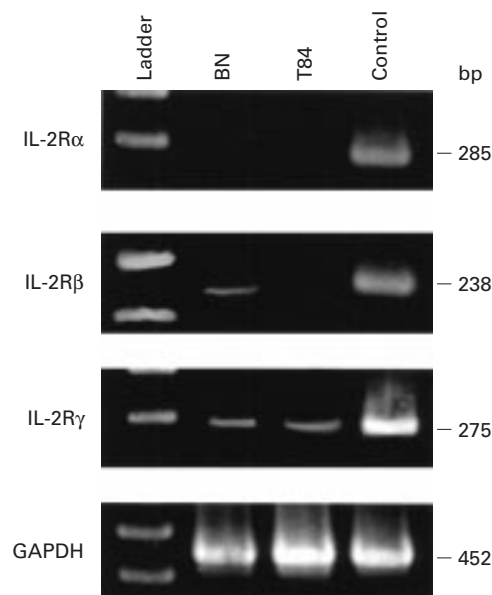


Figure 5 Constitutive expression of interleukin 2 receptor (IL-2R) α , β , and γ chain mRNA genes. Control transcripts were derived from peripheral blood lymphocytes and exhibited all three chains of the receptor. In contrast, BN (small intestinal cells) expressed β and γ chains, and T84 cells γ chain only. bp, base pairs; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

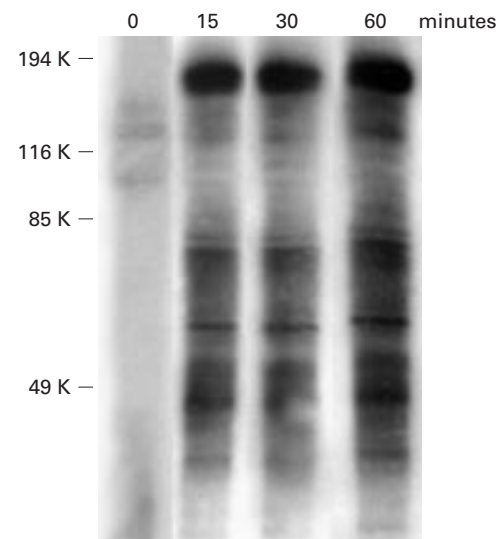


Figure 6 Time dependent tyrosine phosphorylation of proteins in BN cells treated with interleukin 2 (IL-2) 5 U/ml. Numbers on the left indicate molecular weight. Multiple bands of tyrosine phosphorylated proteins were detected by 15 minutes.

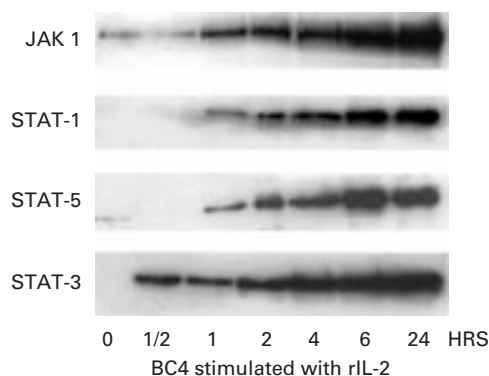


Figure 7 Time dependent increase in expression of janus kinase 1 (JAK1) and signal transducers and activators of transcription (STAT)-1, -3, and -5 in BN cells treated with interleukin 2 (IL-2) 5 U/ml. Cells were treated with IL-2 and lysed at various time points from 0.5 to 24 hours of treatment and proteins detected as described under methods. IL-2 treatment increased expression of all proteins from 30 to 60 minutes post incubation.

and -5 which are all involved in signal transduction of IL-2 (fig 7) (reviewed by Gaffen and colleagues²⁶). Tyrosine phosphorylated proteins appeared within 15 minutes and expression of JAK1 and STAT-3 was increased within 30 minutes of incubation with IL-2, within the time frame of enhancement of secretagogue stimulated secretion.

Discussion

The results of this study provide evidence that IL-2 modulates ion transport in a small intestinal cell line. Previous investigators have identified functional IL-2 receptor in the intestines of rat and human large intestine and in rat small intestinal and human colon cancer cell lines.^{14, 15} IL-2 has been shown to modulate epithelial growth in intestinal cell cultures, transforming growth factor β gene expression in IEC-6 cells, and to activate signal transduction pathways via tyrosine phosphorylation in human colon cancer cell cultures. To date, studies have not assessed the effect of IL-2 on epithelial transport. The small intestinal cells utilised in this study express a functional IL-2 receptor which was activated with IL-2 treatment as evidenced by increases in tyrosine phosphorylation and JAK and STAT proteins. Treatment with IL-2 enhanced secretagogue stimulated chloride secretion and stimulated cellular proliferation but did not affect permeability.

We have developed and characterised novel human small intestinal cell lines which grow in polarised monolayers. Some exhibit vectorial transport.¹⁶ When studied under voltage clamp conditions, these cells exhibit low baseline levels of electrogenic ion transport and when treated with secretagogues such as forskolin develop anion secretion. Incubation of one of the lines, BN, with IL-2 did not alter baseline electrical parameters (that is, electrogenic ion transport) indicating that IL-2 is not a secretagogue. However, low doses of IL-2 caused a 50% or more increase in secretagogue stimulated chloride secretion. Demonstration that CFTR mRNA is present in BN cells, and our previous observation that secretion could be

blocked by the CFTR blocker diphenylamine-2-decarboxylate,¹⁶ suggests that secretion is occurring via CFTR chloride channels. BN cells also exhibit a secretagogue stimulated Isc which is independent of chloride, most likely bicarbonate.¹⁷ Augmentation of chloride secretory capacity is not specific to IL-2 as IL-15, a cytokine which shares the IL-2R β and γ chains and produces similar biological effects to IL-2,¹⁹ also caused a dose dependent increase in secretagogue stimulated anion secretion. However, treatment of the cells with IL-4 had no effect.

It is not clear from our experiments how IL-2 is increasing the capacity for chloride secretion. The IL-2 effect was mediated via the IL-2R as it was blocked by preincubating the cells with a blocking antibody directed against the IL-2R β chain. IL-2 treatment also increased tyrosine phosphorylation¹⁵ and expression of JAK1 and STAT-1, -3, and -5 (which are involved in the signal transduction pathway of IL-2 receptor) all within the time frame of the effect on ion transport. If and how these signals modulate secretion is unknown. While interferon γ inhibits chloride secretion in T84 cells primarily by inhibition of CFTR gene expression²⁷ and the subsequent decrease in apical chloride channels, this mechanism is unlikely to explain the effect on BN cells. Time course studies with the small intestinal cells indicate that the IL-2 effect occurs by 30 minutes to two hours after addition to the media, suggesting that the effect is not caused by altered gene expression or new CFTR synthesis. Other investigators have demonstrated that IL-2 can induce an immediate increase in potassium secretion when applied to the surface of macrophages.²⁸ This is also not likely to explain our results as IL-2 failed to alter baseline electrical parameters. The data imply that IL-2 is affecting the secretory apparatus, for example by insertion of more CFTR into the apical membrane or altering ionic gradients driving chloride efflux, without affecting secretion per se.

The observations are in keeping with other reports of cytokine modulation of epithelial transport. IL-1 and IL-3 stimulate chloride secretion in chicken intestine²⁹ via cyclooxygenase dependent mechanisms. IL-1 treatment in an epithelial-subepithelial myofibroblast coculture model³⁰ also enhanced epithelial responsiveness to secretagogues via myofibroblast secretion of arachidonic acid metabolites such as prostaglandin E₂. In another study, Madsen and colleagues³¹ demonstrated that IL-10 increased sodium and chloride absorption in vitro in rat small intestine and attenuated secretagogue induced chloride secretion. The same investigators observed that IL-10 treatment reduced chloride secretion and inhibited the cytokine induced increase in epithelial inulin permeability in T84 cells.³² Recent reports provide additional evidence that inflammatory mediators may reduce mucosal fluid and electrolyte secretion.^{33, 34} Mucosal hyporesponsiveness to secretagogues has been observed in animal models of TNBS colitis. Increased production of nitric oxide in the inflamed mucosa

accounted for this inhibition of mucosal secretion. Several other interleukin receptors, including IL-4, -7, -9, and -15, have been identified on human intestinal epithelial cells.¹⁵⁻³⁵ Treatment of the cells with these cytokines stimulates signal transduction pathways suggesting a physiological role, although functional studies have not determined the role these cytokines may play. Moreover, IL-15, which shares a common γ chain with IL-2, modulates transepithelial resistance in T84 monolayers³⁵ and induces a similar effect on transport in our small intestinal cells as we reported here for IL-2. Taken together, the available data suggest that the cytokine network can play a role in modulating epithelial transport.

In contrast with the ion transport changes, IL-2 did not affect monolayer permeability, as has been described for some cytokines such as interferon γ and tumour necrosis factor α .¹¹⁻³⁵⁻³⁷ IL-2 treatment stimulated cellular proliferation, consistent with previous observations.¹⁴⁻¹⁵ Ciacci and colleagues¹⁴ observed a biphasic effect of IL-2 on cellular growth in rat IEC-6 cells. Treatment with 10 and 100 U/ml stimulated proliferation at 24 hours but the higher dose caused growth inhibition at 48 hours. BN cells exhibited proliferation at lower levels, less than 1 U/ml. The differences in the studies could be due to the relative amounts of IL-2R β and IL-2R γ c expressed on rat and human cells. While we have not studied the differential surface expression of the various IL-2R chains and their relative binding affinities, such studies have been carried out on human lymphoid cells.³⁸⁻³⁹ CD8⁺ T cells and natural killer cells expressed high levels of IL-2R β but very little IL-2R α , while CD4⁺ T cells expressed very low amounts of IL-2R β . Furthermore, differential expression of these two IL-2R subunits on CD4⁺ and CD8⁺ T cells accounted for the differences in IL-2 responsiveness because $\beta\gamma$ constituted the functional receptor.⁴⁰⁻⁴¹ The combination of β and γ chains is required for signal transduction⁴² and the proliferative response, and both β and γ chains were present in our cell line. The α chain which provides a high affinity ligand for IL-2 does not appear to be essential for the proliferative effect and could not be identified in our small intestinal cells, consistent with previous reports.¹⁴⁻⁴² Interestingly, the dose required in our studies to elicit cellular proliferation was considerably less than that required for stimulation of chloride secretion, suggesting that the two biological effects are elicited by different cellular mechanisms. This effect on epithelial cell proliferation may play an important role in maintaining mucosal integrity in intestinal inflammation.

In conclusion, IL-2 was formerly considered to be a cytokine primarily involved in stimulating the growth of lymphocytes, macrophages, and other immunocytes. Functional IL-2R and an effect of IL-2 on epithelial cell proliferation has been demonstrated in several different cell types. This study provides additional evidence for a role of IL-2 in modulating epithelial function. Rather than stimulating ion secretion per se, IL-2 treatment increases the maximal

secretory capacity of human small intestinal enterocytes in response to known secretagogues. The data imply a possible role for IL-2 in modulating enterocyte proliferation and amplifying cellular secretion in some inflammatory disorders of the intestine.

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