Interferon-γ downregulates ion transport in murine small intestine cultured in vitro

DONGJIN YOO, WINSON LO, STEPHEN GOODMAN, WASIF ALI, CAROL SEMRAD, AND MICHAEL FIELD
Division of Digestive and Liver Diseases, Departments of Medicine and Physiology and Cellular Biophysics, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Received 12 October 1999; accepted in final form 12 June 2000

Yoo, Dongjin, Winson Lo, Stephen Goodman, Wasif Ali, Carol Semrad, and Michael Field. Interferon-γ downregulates ion transport in murine small intestine cultured in vitro. Am J Physiol Gastrointest Liver Physiol 279: G1323–G1332, 2000.—Effects of IFN-γ on mammalian small intestinal ion transport were studied in vitro using incubated sheets of murine small intestine in Ussing chambers. In oxygenated standard culture medium containing hydrocortisone and antibiotics, they maintained their short-circuit current (Isc) responses to glucose and theophylline by 48 h. Histological examination revealed a 50% diminution of villus height over 36 h but no change in crypts. Height was better maintained during a 36-h incubation of small intestine from SCID mice, suggesting a role for B or T lymphocytes in villus atrophy. Exposure of small intestine to 100 U/ml IFN-γ SCID mice, suggesting a role for B or T lymphocytes in villus maintained during a 36-h incubation of small intestine from SCID mice, suggesting a role for B or T lymphocytes in villus atrophy. Exposure of small intestine to 100 U/ml IFN-γ for 36 h decreased basal Isc by 40% and Isc responses to glucose and theophylline by ~70%; at 1,000 U/ml for 36 h, IFN-γ inhibited these Isc responses by 90%. An inhibitor of inducible NO synthase did not reverse these effects, suggesting that they are not mediated by NO. Tissue resistance, mucosal K+ content, and epithelial morphology were not affected. Ouabain-sensitive ATPase activity in homogenates was inhibited 60% by IFN-γ (100 U/ml for 36 h). IFN-γ inhibition of basal Isc responses to glucose and theophylline also occurred in SCID mouse small intestine. Thus murine small intestinal sheets can be maintained viable in vitro for at least 48 h, although villus blunting develops (but less so in SCID mouse small intestine). Also, prolonged exposure to IFN-γ downregulates Na+-coupled glucose absorption, active Cl− secretion, and Na+-K+-ATPase activity, effects unlikely to be mediated by enhanced NO.

To effectively study interactions between the intestinal immune system and the intestinal epithelium, an intact epithelial preparation that can maintain its transport and barrier properties for an extended period of time is needed. In T84 cells, for example, interferon-γ (IFN-γ)-induced phenotypic changes require 24–48 h to manifest themselves (6). Studies of such slow-to-appear cytokine effects have been accomplished with colon cancer cell lines such as T84 but not heretofore with normal intestine. To accomplish the latter, we have developed an in vitro method for maintaining murine small intestine viable and intact for at least 48 h.

We selected IFN-γ as the cytokine for initial study because of its central role in inflammation and because its epithelial effects had previously been studied in T84 cells (6). T helper-1 (Th1) lymphocytes, when activated, secrete IFN-γ (31), and Crohn’s disease, the panenteric, transmural subtype of inflammatory bowel disease, is characterized by a Th1 cell cytokine response (4, 30, 34, 37). A Th1 cell response is also characteristic of celiac disease (gluten-sensitive enteropathy), in which there is small intestinal malabsorption associated with villus atrophy and crypt hyper trophy (20). In colon cancer cell lines, IFN-γ has been shown to upregulate major histocompatibility complex (MHC) class I and II expressions, enhance neutrophil adhesion, downregulate cAMP-activated Cl− secretion, and attenuate intestinal barrier function, enhancing the absorption of macromolecules (6, 42). Whether this so-called “phenotypic switch” caused by IFN-γ in colon cancer cell lines also occurs in normal mammalian small intestine in primary culture has not heretofore been determined.

In this study, we developed a primary culture system for sheets of murine small intestine large enough to place in Ussing chambers, and using this system, we demonstrated that IFN-γ downregulates both Na+-coupled glucose absorption, which arises in villus cells, and cyclic nucleotide-activated active Cl− secretion, which arises mainly in crypt cells. It also inhibited Na+-K+-ATPase activity, as measured in homogenates. These ion-transport changes developed without significant changes in epithelial morphology or K+ content.

METHODS

Primary organ culture. Two- to four-month-old mice of CD-1 strain, obtained from Charles River Laboratories (Wilmington, MA), were gavaged with a mixture of 1.5 mg met-
ronidazole and 7.5 mg neomycin, fasted, except for water, for 12–18 h, and then killed by cervical dislocation. The small intestine, from the ligament of Treitz to the ileocecal junction, was rapidly excised, immersed in oxygenated cold normal saline, cut open longitudinally with a fine iridotomy scissors, rinsed with normal saline to remove intestinal contents, and then transferred to cold bicarbonate-buffered Ringer solution gassed with 95% O$_2$–5% CO$_2$. Sections 2-cm long were placed, mucosa up, on a polyester mesh screen (Spectrum, Laguna Hills, CA), which was positioned over pins set into a circular plastic frame surrounding a curved end-rectangular opening measuring 1.6 cm in length and 0.4 cm in width (Fig. 1). The lateral margins of the intestine were stretched to fit over the pins. This preparation was then placed in a 6-cm diameter petri dish (Fisher Scientific, Springfield, NJ) to which 13 ml of culture medium were added, creating a level just covering the tissue surface. In some experiments (those for tissue K$^+$ and Na$^+$-K$^+$-ATPase measurements), the muscle layers were stripped off before mounting tissues.

The culture medium consisted of bicarbonate-buffered Ringer solution to which the following were added: 0.5 μg/ml FeSO$_4$-7H$_2$O, 0.08 μg/ml MnSO$_4$·H$_2$O, 0.15 μg/ml ZnSO$_4$·7H$_2$O, 0.25 μg/ml CuSO$_4$, MEM vitamins and amino acids (Life Technologies, Grand Island, NY), insulin-transferin-selenium (Life Technologies), 20 mM fructose, 5 mM L-glutamine (Life Technologies), 0.8 μg/ml hydrocortisone, 300 μg/ml ascorbate, 0.2 μg/ml vitamin B$_{12}$, 15 μg/ml glutathione, 15 μg/ml phenol red, 10% fetal bovine serum (Life technologies), 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies). Murine recombinant IFN-γ (Genzyme, Cambridge, MA) was added to some cultures. The loaded petri dishes were placed in the bases of loosened, 120-ml containers (Allegiance, Montgomery, NY), which were set on a slowly rotating platform and gassed with a mixture of 95% O$_2$–5% CO$_2$ for 30 min. The containers were then screwed shut, and their interphases were sealed with oil. Then the containers were placed on a slowly rocking shaker in an incubator at 37°C for varied time intervals. Unless otherwise specified, materials were purchased from Sigma Chemical (St. Louis, MO).

**Electrical measurements.** The murine intestinal explants were clamped into Ussing chambers with tissue openings of the same size as specified above for the frames used for tissue incubation. Short-circuit currents, potential differences, and tissue resistances were determined with automatic voltage clamps, which corrected for fluid resistances.

**Morphological studies.** Tissue explants, taken either directly from incubation or from Ussing chambers, were placed in a gelatin capsule (Polysciences, Warrington, PA) containing OCT (Sakura Finetek, Torrance, CA), gradually frozen in a dry ice-acetone mixture, and then stored at −70°C until further processing. Sections (5 μm) were sliced by cryostat at −10 to −20°C, placed on slides, and stained with hematoxylin and eosin. Measurements of villus height and width, crypt depth, and number of villi and crypts per 100-fold magnified high-power field were made on photographs of these images. Measurements were made on a minimum of three villi per image, and results from multiple images were averaged. Because longitudinally cut crypts were less frequent, 2–4 crypt lengths were measured for each mouse. Small intestines from three mice were used for histological evaluation. Intestinal sheets from each of the mice were first tested in Ussing chambers and shown to respond normally to IFN-γ, glucose, and theophylline.

**Tissue K$^+$ measurements.** For K$^+$ determinations, exposed sections of muscle-stripped intestinal explants were cut out and dried at −80°C overnight, weighed, and extracted in equal volumes of glacial acetic acid and 3 M TCA. The extracts were sonicated until clear, diluted with two volumes of distilled water, and centrifuged. K$^+$ concentrations in the supernates were then determined by atomic absorption spectroscopy. As a control for this method of intracellular K$^+$ assay, K$^+$ concentration was determined in mucosae incubated at 37°C for 3 or 5 h with 10 mM ouabain and 15 μg/ml amphotericin B and compared with concentrations in mucosae incubated for the same time periods without addition of ouabain and amphotericin.

**Na$^+$-K$^+$-ATPase measurement.** Na$^+$-K$^+$-ATPase activity was measured as ouabain-inhibitable phosphate production from ATP by a modification of prior methods (1, 2, 32). Briefly, mucosa stripped of muscle was homogenized at 650 rpm with 20 vertical strokes for 2 min. The homogenization medium contained 250 mM sucrose, 30 mM histidine, and 1 mM EGTA (pH 7.2). Tissues were washed with normal saline and then twice with each of two EDTA-containing Tris-buffered solutions (pH 7.2), the first 5 mM EDTA in 50 mM Tris and the second 1 mM EDTA in 50 mM Tris. The homogenates were then incubated for 1 h at 4°C with SDS (4.8 nmol/mg protein), following which they were centrifuged at 750 g for 3 min to remove cellular debris. Aliquots (120 μl) were then added to 1.1 ml of prewarmed reaction medium (130 mM NaCl, 20 mM KCl, 5 mM MgCl$_2$, 3 mM Na$_2$SO$_4$, and 30 mM Tris, pH 7.2) and incubated with or without 10 mM ouabain for 30 min. ATP (final concn, 3 mM) was then added, and the incubation continued for an additional 15 min. The assay temperature was 37°C. Free phosphate was measured in a spectrophotometer at 700 nm. Enzyme activities were
expressed as micromoles of phosphate liberated per milligram of protein per hour.

Statistical analysis. $I_{sc}$ differences, crypt depths on histological sections, and enzyme activities were analyzed by Student’s $t$-test, tissue $K^+$ concentrations by ANOVA, and villus height and width by the Kruskal-Wallis test for significance. All results are presented as means ± SE.

RESULTS

Effects of incubation time on ion transport and resistance. $I_{sc}$ responses to both glucose and theophylline were not significantly affected by incubation time up to 36 h compared with responses in freshly removed tissues not incubated in vitro (Fig. 2). At 48 h, the glucose response was still unchanged from the time 0 response but the theophylline response had declined by ~40% [$P < 0.02$; at 36 h, the mean theophylline response had declined by 28% but this change is not statistically significant ($P > 0.2$)]. Thus sheets of murine small intestine can be incubated in vitro for an appreciable number of hours without loss of either sodium-coupled glucose absorption or theophylline-stimulated active $Cl^-$ secretion. In a single experiment, $I_{sc}$ responses were tested after 5 days of incubation in vitro: both glucose and theophylline responses were in excess of 175 $\mu$A/cm$^2$.

Tissue resistance declined with incubation time. In six mice, resistance declined by 38% over 36 h (from $27.1 \pm 2.46 \text{ } \Omega \cdot \text{cm}^2$ at $t = 0$ to $16.9 \pm 1.62 \text{ } \Omega \cdot \text{cm}^2$ at $t = 36$ h; $P < 0.03$). Because measurements were made on unstripped tissue, it is uncertain whether this decline in resistance arose from epithelial changes, smooth muscle changes, or both.

Effects of incubation time on histology and $K^+$ content. Histological sections made from both fresh (unincubated) intestine and intestine incubated in vitro for 36 h are shown in Fig. 3. Measurements of mean villus heights and widths at both times are shown in Table 1. An ~50% decrease in villus height developed over the 36-h incubation period, whereas villus width did not change. Examination of the crypt region indicates no
Table 1. Effects of incubation time and IFN-γ on villus height and width

<table>
<thead>
<tr>
<th>Mouse</th>
<th>36-h Incubation</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No Incubation</td>
<td>No IFN-γ</td>
<td>IFN-γ</td>
<td></td>
</tr>
<tr>
<td>Height, μm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>106.5 ± 1.3</td>
<td>68.7 ± 2.0</td>
<td>54.2 ± 1.7</td>
</tr>
<tr>
<td>2</td>
<td>156.9 ± 2.8</td>
<td>57.3 ± 1.6</td>
<td>47.5 ± 1.6</td>
</tr>
<tr>
<td>3</td>
<td>117.3 ± 1.9</td>
<td>57.4 ± 1.7</td>
<td>54.9 ± 1.8</td>
</tr>
<tr>
<td>Mean</td>
<td>126.9 ± 15.3</td>
<td>61.1 ± 3.8</td>
<td>52.2 ± 2.4</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8–27 histological sections for each of 3 mice. Villus height at 36 h is significantly less than in unincubated tissues (P < 0.001). There are no significant differences at 36 h between results for interferon-γ (IFN-γ)-treated and control tissues.

significant change in crypt histology or diminution in crypt number over the 36-h incubation period. The number of crypts divided by the number of villi was 2.29 ± 0.21 at t = 0 and 2.08 ± 0.13 at t = 36 h. Examination of crypt depth in a limited number of crypts cut longitudinally indicated no change over 36 h at t = 0, 49.3 ± 1.21 μm (n = 9); control at t = 36 h, 53.0 ± 3.98 μm (n = 9); IFN-γ treated at 36 h, 46.4 ± 2.44 μm (n = 8); no statistically significant differences.

As an additional measure of epithelial viability, we determined mucosal K⁺ content (μmol/mg dry wt), comparing values at t = 0 and t = 36 h. We used muscle-stripped intestine for these determinations to avoid contamination from the K⁺ content of smooth muscle. We first tested the suitability of the stripped preparation by measuring its Iₛₑ responses after 36 h incubation: means and ranges of Iₛₑ responses for stripped tissues from three mice were 346 μA/cm² (range 135–527) for glucose and 246 μA/cm² (range 173–381) for theophylline. Clearly, vigorous Iₛₑ responses can be elicited from stripped intestine after prolonged incubation in vitro. The K⁺ contents of stripped tissues, both those incubated for 36 h and those tested without preincubation, are compared in Table 2. The two values do not differ significantly.

Effects of IFN-γ on ion transport and resistance. Prolonged incubation of tissues with IFN-γ diminished both baseline Iₛₑ and Iₛₑ responses to glucose and theophylline. Baseline Iₛₑ measured ~15 min after mounting tissues in vitro and just before mucosal-side addition of glucose, was decreased by IFN-γ (100 U/ml) by ~40% (Table 3). The time course of the effect of IFN-γ on Iₛₑ responses to glucose and theophylline is shown in Fig. 4. At 100 U/ml IFN-γ, the magnitude of inhibition progressed from 24 to 36 h and, at least with respect to the glucose response, from 36 to 48 h. At 1,000 U/ml, inhibitions of Iₛₑ responses were greater than at 100 U/ml. Inhibition of Iₛₑ responses at concentrations of IFN-γ from 25 to 1,000 U/ml are compared in Table 4. No significant differences in percentage inhibition from 25 to 500 U/ml were found, although, as also noted in Fig. 4, inhibition was greater at 1,000 U/ml. The present results for time course and dose dependency are similar to those obtained by Colgan et al. (6), using T84 cell monolayers.

In contrast to the results of Colgan et al. (6), however, tissue resistance was unaffected by IFN-γ: in tissues from six mice incubated for 36 h in either the presence or absence of 100 U/ml IFN-γ, resistances in control and IFN-γ-treated tissues were identical (16.9 ± 1.62 vs. 16.8 ± 1.53 Ω·cm²). It should be noted, however, that the baseline resistance of the T84 cell monolayers was in the 600–800 Ω·cm² range. Because of the much greater leakiness of murine small intestine, a change in cellular or paracellular resistance properties brought on by IFN-γ may have been missed. During prolonged incubation in vitro, there may be significant cell desquamation. This would produce holes of several micrometers in diameter and would likely outweigh the effect of any change in tight junction permeability.

The inhibitory effect of IFN-γ on Iₛₑ responses to glucose and theophylline could represent, as previously suggested for T84 cells (6), a shift in phenotype: down-regulation of ion and nutrient transport function and upregulation of other functions more related to the immune system and inflammation. Alternatively, it could represent a loss of epithelial viability. To exclude
the latter, the effects of IFN-γ on tissue morphology and K⁺ content were evaluated. Morphological characteristics are shown in Fig. 3 and Table 1 and K⁺ content in Table 2. It can be seen that incubation for 36 h in the presence of IFN-γ (100 U/ml) did not significantly affect either. Thus tissue viability does not appear to have been compromised by prolonged incubation with IFN-γ.

Absence of a role for nitric oxide in ion transport inhibition by IFN-γ. In murine proximal tubule epithelial cells, IFN-γ, in combination with lipopolysaccharide, inhibits Na⁺-K⁺-ATPase activity by a nitric oxide (NO)-mediated mechanism [i.e., the NO synthase (NOS) inhibitor \(\text{N}^{\text{G}}\)-nitro-L-arginine prevented this inhibition] (18). To evaluate whether the ion-transport effects of IFN-γ were secondary to upregulation of NOS by IFN-γ, we added the inducible NOS inhibitor \(\text{N}^\text{G}\)-nitro-L-arginine methyl ester (\(\text{L}\)-NAME) to incubations with IFN-γ. Results are shown in Table 5. \(\text{L}\)-NAME did not even slightly reduce the effect of IFN-γ. Although there is some arginine in the incubation medium (0.36 mM), the concentration of \(\text{L}\)-NAME was 14-fold greater and \(\text{L}\)-NAME is reported to have an at least fivefold greater affinity for NOS than does arginine (19).

Effect of IFN-γ on Na⁺-K⁺-ATPase activity. INF-γ has been shown in T84 cells to downregulate several epithelial transport proteins including Na⁺-K⁺-ATPase (6, 37a). Because the Na pump is involved in both Na-coupled glucose transport and theophylline-stimulated Cl⁻ secretion, we determined the effect of 36 h incubation with IFN-γ on Na⁺-K⁺-ATPase activity. Enzyme activity was assayed in homogenates of muscle-stripped small intestine as ouabain-sensitive phosphate production from ATP as described in METHODS. Results are shown in Table 6. Although mean ouabain-sensitive ATPase activity was 38% less in 36-h incubated control mucosa than in unincubated mucosa, this difference was not statistically significant (\(P > 0.3\)). Incubation with IFN-γ for 36 h inhibited ouabain-sensitive ATPase activity by ~60%, which compares fairly closely to the percentage inhibitions of \(I_{sc}\) responses to both glucose and theophylline (~70%).

### Table 4. \(I_{sc}\) responses to glucose and theophylline at different IFN-γ concentrations

<table>
<thead>
<tr>
<th>IFN-γ Concentration, U/ml</th>
<th>Responses to Glucose</th>
<th>Responses to Theophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\Delta I_{sc}) ((\mu\text{A/cm}^2))</td>
<td>(\Delta I_{sc}) ((\mu\text{A/cm}^2))</td>
</tr>
<tr>
<td>No IFN-γ</td>
<td>IFN-γ</td>
<td>IFN-γ/No IFN-γ, %</td>
</tr>
<tr>
<td>25</td>
<td>87 ± 2.1</td>
<td>37 ± 13.8</td>
</tr>
<tr>
<td>50</td>
<td>134 ± 46.5</td>
<td>53 ± 19.3</td>
</tr>
<tr>
<td>100</td>
<td>152 ± 30.2</td>
<td>42 ± 13.2</td>
</tr>
<tr>
<td>500</td>
<td>196 ± 26.0</td>
<td>42 ± 24.4</td>
</tr>
<tr>
<td>1,000</td>
<td>141 ± 15.6</td>
<td>7 ± 01.9</td>
</tr>
</tbody>
</table>

Values are means ± SE for 2–5 mice. Tissues were incubated for 36 h in absence or presence of 100 U/ml IFN-γ. There are no significant differences for %inhibition between 25 and 500 U/ml IFN-γ, but inhibition at 1,000 U/ml differs from that of other groups (\(P < 0.01\)).
Table 5. Failure of \( L^-\text{NAME} \) to reverse downregulation of \( I_{sc} \) responses by IFN-\( \gamma \)

<table>
<thead>
<tr>
<th>Condition</th>
<th>( n )</th>
<th>( \Delta I_{sc} (\mu A/cm^2) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>+Glucose: 161.9 ± 36.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Glucose: 169.7 ± 20.60</td>
</tr>
<tr>
<td>IFN-( \gamma )</td>
<td>5</td>
<td>+Theophylline: 23.7 ± 0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Theophylline: 39.7 ± 0.74</td>
</tr>
<tr>
<td>IFN-( \gamma )+( L^-\text{NAME} )</td>
<td>5</td>
<td>16.0 ± 0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. \( I_{sc} \) responses in IFN-\( \gamma \)-treated tissues are significantly less than in control tissues \((P < 0.01)\). IFN-\( \gamma \) concentration was 100 U/ml, and \( N^-\text{nitro-L-arginine methyl ester (L-NAME)} \) concentration was 5.0 mM. L-NAME alone, in the absence of IFN-\( \gamma \), had no effect, yielding results indistinguishable from control.

Effects of IFN-\( \gamma \) in SCID mice. Because the intestinal mucosa contains an appreciable number of leukocytes and other mesenchymal cells, it remains uncertain whether the effects of the added IFN-\( \gamma \) were exerted directly on enterocytes or only indirectly via another cytokine released from mesenchymal cells. Activated T cells, for example, release a number of cytokines that directly or indirectly may affect ion transport [in addition to IFN-\( \gamma \), tumor necrosis factor-\( \alpha \), interleukin-2 (IL-2), IL-4, IL-5, IL-10, and IL-13; Ref. 31]. To provide a partial answer to this question, we tested the effect of IFN-\( \gamma \) on the small intestine of severe combined immunodeficiency disease (SCID) mice since they are devoid of mature T and B lymphocytes (25). In this preparation, IFN-\( \gamma \) (100 U/ml for 36 h) inhibited \( I_{sc} \) responses to glucose and theophylline by ~60% (Fig. 5), suggesting that the observed in vitro effects of IFN-\( \gamma \) were not mediated by mucosal T or B lymphocytes. It is also of interest, as shown in Table 3, that the basal \( I_{sc} \) of SCID mouse small intestine is lower than that of CD-1 mouse small intestine and is unaffected by IFN-\( \gamma \).

Figure 6 shows the morphology of SCID mouse intestine incubated for 36 h with or without IFN-\( \gamma \). It can be seen that IFN-\( \gamma \) did not significantly alter intestinal morphology in SCID mice. Figure 6 does reveal one surprising finding: After a 36-h incubation, villus height in the SCID mouse intestine shown is clearly greater than in normal intestine. This result is borne out by the histology of two additional incubated SCID mouse small intestinal preparations. We also examined the histology of two unincubated \((\text{time } 0)\) SCID mouse small intestinal mucosae. Villus height appeared to be well within the range of values for CD-1 mouse small intestine at \( \text{time } 0 \). We did not attempt to quantitate villus height in SCID mouse small intestine because the \( n \) was small and there is significant variability in height along the length of intestine from proximal jejunum to distal ileum. Thus the greater villus height in 36-h incubated SCID mouse small intestine appears to be due to less villus blunting and not to a greater villus height at \( \text{time } 0 \). Villus height in normal (CD-1 mouse) small intestine appears, therefore, to diminish during prolonged incubation in vitro due to one or more factors absent from SCID mouse intestine. The factors that come to mind are mucosal lymphocytes and enteric microorganisms (SCID mice are raised and maintained in a relatively germ-free environment).

DISCUSSION

Primary culture of mammalian small intestine. We have developed a culture system for intact sheets of murine small intestine that maintains viability for at least 48 h. The exposed area of these epithelial sheets (1.6 cm \( \times \) 0.4 cm), together with the surrounding pinned area, was large enough to mount in Ussing chambers specially designed for elongated tissue sections. Cultured tissues maintained their \( I_{sc} \) responsiveness to glucose (reflecting Na\(^+-\)coupled glucose absorption, a villus cell function) and theophylline (reflecting active Cl\(^-\) secretion, largely a crypt cell function). Both muscle-stripped and full-thickness intestine could be maintained in this way. Despite maintaining these absorptive and secretory functions at their nonincubation rates, cultured intestine underwent morphological change, villi being 50% shorter at 36 h than at \( \text{time } 0 \) without evident changes in crypts. Furthermore, electrical resistance decreased by ~40% over 36 h of incubation in vitro; this may have been due to shedding of epithelial cells during incubation.

It is intriguing that villus height diminished by 50% over 36 h without any reduction in the \( I_{sc} \) response to glucose. This apparent discrepancy can perhaps be explained by the results of a prior study (9) in which the surface location of the Na\(^+-\)coupled glucose transporters was determined in rat ileum. Using \(^{3}\)Hjphlorizin as a label, these transporters were found bunched at the tips of villi and only minimally present along the sides of villi. In contrast, in the ileum of rats rendered diabetic with streptozocin (glucose absorption is enhanced in experimental diabetes), the glucose transporters were found spread evenly over the entire villus surface, sides as well as tips. Whether these observations on rat ileum apply also to mouse small intestine and jejunum as well as ileum is uncertain. Certainly in vitro changes in cell proliferation and apoptosis could also influence the proportion of intestinal epithelial cells engaged in glucose absorption. Whatever the explanation, our results do demonstrate that there is not a tight correlation between villus surface area and glucose absorptive capacity. We are
unaware of any evidence that glucose absorption is diminished in celiac disease, for example.

Primary culture of intact small intestine for periods up to 48 h has been reported previously (10, 16, 28, 43) for rabbit, mouse, and human small intestine with good, although not perfect, preservation of brush-border enzyme activities, cellular functions, and epithelial structure. As in the present study, some villus blunting has been noted. Fetal small intestine appears to be easier to maintain in vitro, survival for multiple days having been accomplished (26). In all of these studies, the sizes of incubated tissue sections were quite small, mostly \(1 \text{mm}^3\). To our knowledge, this is the first published report of maintaining in vitro for up to 48 h small intestinal sections large enough to mount in Ussing chambers for measurements of transepithelial electrical properties (microchambers for biopsies would enable use of smaller mucosal sections, but these chambers are very difficult to use). We believe that a key element in the prolonged survival of these tissues in vitro was the stretch imposed in pinning opened intestine onto frames. Through stretch, better oxygenation of intervillus spaces and crypts was likely provided. Stretch has also been shown to increase proliferation of fetal rat lung epithelial cells (21) and to activate immediate early genes such as \(c\)-fos, \(c\)-jun, \(c\)-myc, JE, and EGR-1 in cardiac muscle cells (36). These immediate early genes, if activated in the intestine by stretch, could act as promoters of enterocyte proliferation.

The reasons for the development of villus atrophy during prolonged in vitro incubation remain to be established. Of interest is a report by MacDonald and Spencer (23) showing that human fetal small intestine in organ culture develops villus atrophy and crypt hypertrophy when stimulated with T cell activators (PWM or anti-CD3 antibody) and that these changes could be prevented by addition of cyclosporin A, which prevents T cell activation. Some T cell activation may have occurred spontaneously in our culture system. In future experiments, we will explore the effect of cyclosporin A and the role of Th1 cell cytokines on the villus atrophy that we have observed.

**Effects of IFN-\(\gamma\).** Incubation with IFN-\(\gamma\) for 24–48 h downregulated short-circuit responses to both glucose and theophylline and decreased basal \(I_{sc}\), producing these effects without significantly altering tissue resistance, morphology, or \(K^+\) content. The time course and concentration dependence of these effects were similar to those previously observed for T84 cells (6). The inhibitory effect of IFN-\(\gamma\) was seen in SCID as well as in CD-1 mice, suggesting that its intestinal epithelial effects were not indirectly exerted via a primary effect on T or B lymphocytes.

IFN-\(\gamma\) has been shown previously to downregulate ion transport or, more specifically, active \(Cl^-\) secretion in the colon cancer cell lines T84 (3, 6) and HT-29 (3) and in a cystic fibrosis transmembrane conductance regulator (CFTR) gene rescued pancreatic cancer cell line derived from a patient with cystic fibrosis (CF-PAC) (22). Downregulation of the apical \(Cl^-\) channel, CFTR, has been demonstrated at functional, mRNA, and protein levels (3, 6, 22). Downregulation of the \(Na^+-K^+\)/\(2Cl^-\) cotransporter and \(Na^+-K^+\)/ATPase has also been shown (6). The present study, in addition to demonstrating the previously observed inhibition of active \(Cl^-\) secretion, extends the transport effects of IFN-\(\gamma\) to include \(Na^+\)-coupled sugar absorption. Thus IFN-\(\gamma\) interferes with transport functions in both villus and crypt cells.

In T84 cells, IFN-\(\gamma\) decreases transepithelial resistance and increases permeability to mannitol and inu-
The effect of IFN-γ on luminal uptake of macromolecules and on the prevalence of proteins involved in the regulation of tight junction permeability, such as ZO-1 (44), will need to be examined.

In murine small intestine, IFN-γ not only decreases the $I_{sc}$ responses to glucose and theophylline but also the basal $I_{sc}$. In large part, this basal current is due to spontaneous secretion of $\text{Cl}^{-}$ and $\text{HCO}_3^{-}$. This has been shown in a study (12) of jejunal ion transport in cystic fibrosis gene knockout mice. Small intestines from these mice, which lack a functional apical membrane anion channel (CFTR), exhibit almost no basal $I_{sc}$. The IFN-γ-induced decrease in the basal $I_{sc}$ of small intestine from CD-1 mice may therefore reflect its overall inhibition of anion secretion and is consistent with its reported downregulation of CFTR in other systems (3, 6). Ileal $\text{HCO}_3^{-}$ secretion, like $\text{Cl}^{-}$ secretion, is electrogenic (27) and probably mediated by CFTR (12). It is interesting to note that the basal $I_{sc}$ in SCID mice was lower than in CD-1 mice and was not significantly decreased by pretreatment with IFN-γ. The lower basal or spontaneous secretion in intestine from SCID mice suggests a role for T or B lymphocytes in the regulation of basal secretion.

The observed inhibitions of ion transport by IFN-γ were not the result of IFN-γ-induced epithelial damage or death since no significant differences in either morphology or epithelial $K^+$ content between IFN-γ-treated (100 U/ml for 36 h) and control intestine were found. However, both villus height and epithelial $K^+$ content were not the result of IFN-γ-induced decreases in $I_{sc}$ due to apical membrane proteins, and the organization of actin and other cytoskeletal proteins in the apical region of the enterocyte remain to be examined. IFN-γ has been shown to disrupt actin organization in the apical cytoplasm of the T84 cell (44). Possible changes in microvillus membrane or apical cytoskeletal properties could be another cause for the inhibition of transport by IFN-γ. The effects of IFN-γ on the appearance of the microvillus membrane on electron microscopy, the density of a few characteristic microvillus membrane proteins, and the organization of actin and other cytoskeletal proteins in the apical region of the enterocyte remain to be examined. IFN-γ has been shown to induce apoptosis in several cell lines/tissues, including HT-29 cells and normal human colon. Other studies suggest, however, that it does not induce apoptosis by itself but only in conjunction with other factors (8).

Possible changes in microvillus membrane or apical cytoskeletal properties could be another cause for the inhibition of transport by IFN-γ. The effects of IFN-γ on the appearance of the microvillus membrane on electron microscopy, the density of a few characteristic microvillus membrane proteins, and the organization of actin and other cytoskeletal proteins in the apical region of the enterocyte remain to be examined. IFN-γ has been shown to disrupt actin organization in the apical cytoplasm of the T84 cell (44).

Examination of all intestinal ion transport processes downregulated by IFN-γ is beyond the scope of the present study. To gain some insight, however, we determined the effect of the cytokine on Na$^+$-$K^+$-ATPase activity, because active Na$^+$ transport is required for $\text{Cl}^{-}$ secretion as well as for Na$^+$-absorptive processes. We found 60% inhibition (Table 6) in a 36-h incubation with 100 U/ml of IFN-γ. In comparison, this concentration of IFN-γ for this time period inhibited glucose-
theophylline-stimulated \( I_{sc} \) responses by closer to 70%, but this difference is not significant. It is worth noting that neither glucose nor theophylline alone or in combination maximally stimulate Na\(^+\) pump activity (the \( I_{sc} \) changes these two agents induce arise from different cells). For example, in control tissues, the addition of alanine subsequent to glucose causes an additional large increase in \( I_{sc} \) (data not shown). Because Na\(^+\)-K\(^+\)-ATPase activity is not rate limiting for individual processes in murine small intestine, IFN-\(\gamma\) very likely causes independent downregulation of additional transport proteins. In colon cancer cell lines, CFTR, Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransport, NHE2, and NHE3 have all been shown to be downregulated by IFN-\(\gamma\) (3, 6, 33, 37a), but these effects could be secondary to a primary effect of IFN-\(\gamma\) on Na\(^+\)-K\(^+\)-ATPase (close to 100% inhibition); ouabain, for example, downregulates at least some of these proteins (37a). In the rat, NHE2 and NHE3 have been shown at both protein and mRNA levels to also be downregulated by IFN-\(\gamma\) (33).

Sundaram and co-workers (38–41) have shown in a rabbit chronic intestinal inflammation model that the activities of a variety of transporters, including Na\(^+\)-glucose cotransport, are altered, usually downward, although constitutive Na\(^+\)/H exchange in crypt cells was found to be enhanced. In contrast to most of these findings (38–41), Na\(^+\)-K\(^+\)-ATPase activity was only slightly inhibited. The inflammation caused was most likely Th1 in type, meaning that there was enhanced production of IFN-\(\gamma\), but other cytokines were also generated, and it is not clear that the several transport changes documented (38–41) were due to IFN-\(\gamma\) alone.

The transport effects of IFN-\(\gamma\) described in the present study are unlikely to be mediated by NO since l-NAME did not prevent the effect of IFN-\(\gamma\) on \( I_{sc} \) responses to glucose and theophylline. Whether NO mediates the IFN-\(\gamma\)-induced inhibition of Na\(^+\)-K\(^+\)-ATPase in small intestine, as noted in proximal tubule cells (13), is a separate issue, untested in the present study.

The genes activated by IFN-\(\gamma\) that are involved in the downregulation of transport proteins such as CFTR, Na\(^+\)-K\(^+\)-ATPase, and perhaps also the Na\(^+\)-glucose cotransporter remain to be established. In several cell lines, including HT-29 cells, IFN-\(\gamma\) has been shown, via activation of STAT-1, to induce the cyclin-dependent kinase inhibitor p21, with a resulting inhibition of cell proliferation (5). In addition to its effect on cell cycle control, p21 has also been shown in mouse keratinocytes to inhibit terminal differentiation of these cells (7). In intestine, p21 may play a similar role because it is highly expressed in postmitotic cells adjacent to the proliferative compartment but less so at later stages of differentiation (11). Na\(^+\)-glucose cotransport function, which has been shown to localize to the villus tip region in rabbit ileum (9), is certainly representative of terminally differentiated villus enterocytes. It is less clear if Cl\(^-\)-secreting cells, most of which appear to reside in crypts, can be considered to be terminally differentiated. Some members of the crypt cell population (e.g., Paneth cells) are not progenitors of villus cells and may be terminally differentiated. With light microscopy, evidence of IFN-\(\gamma\)-induced dedifferentiation was not apparent, but this is a relatively crude measure of cell differentiation. It is of interest, in this regard, that Kerneis et al. (17) showed that coculture of Peyer’s patch-derived lymphocytes with Caco-2 cells (another colon cancer cell line) results in the acquisition by the Caco-2 cells of M-cell-like properties. At least some of this effect was not due to direct contact between lymphocytes and enterocytes but to a soluble factor released by the lymphocytes, possibly IFN-\(\gamma\). Induction of p21 synthesis by IFN-\(\gamma\) could play a role in this transformation.

In conclusion, sheets of murine small intestine suitable for mounting in Ussing chambers can be maintained in vitro for 36–48 h without decreases in Na\(^+\)-dependent glucose absorption or active Cl\(^-\) secretion. Despite maintenance of glucose absorption, a 50% diminution of villus height was observed in small intestine from normal mice (much less so in SCID mouse intestine), indicating a lack of close correlation between abundance of Na\(^+\)-glucose cotransporters and villus surface area and suggesting a role for T or B lymphocytes in the observed partial villus atrophy. IFN-\(\gamma\) downregulates Na\(^+\)-dependent glucose absorption, active anion secretion, and Na\(^+\)-K\(^+\)-ATPase activity without significantly altering epithelial cell viability as determined by mucosal histology and K\(^+\) content. The transport effects of IFN-\(\gamma\) were not reversed by an inducible NOS inhibitor, suggesting that these effects were not mediated by NO. The effect of IFN-\(\gamma\) on tissue resistance previously noted in T84 colon cancer cell monolayers could not be demonstrated in murine small intestine perhaps because of its low baseline resistance.

We thank Professor Joseph Graziano and Vesna Slavkovich for assaying K\(^+\) in our tissue samples.

This study was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Postdoctoral Training Grant DK-07715 (W. Lo) and in part by a gift from Dr. and Mrs. Bernard German in memory of Mrs. German’s brother, Daniel V. Kimberg, M.D., former Chairman of the Department of Medicine at Columbia-Presbyterian Medical Center (W. Lo).

REFERENCES


