Interferon-γ Directly Affects Barrier Function of Cultured Intestinal Epithelial Monolayers

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Abstract

Although epithelia, which often are in intimate contact with lymphoid cells, may bear receptors for various cytokines, it is unclear whether cytokines directly effect epithelial function. We examine the effects of the cytokine interferon (IFN) on barrier function of cultured monolayers of the T_{84} human intestinal epithelial cell line. γIFN, in concentrations and exposures required to show its other biological effects, directly affects such monolayers. Monolayer resistance is substantially diminished by γIFN. Such effects were not due to cytotoxicity as judged morphologically and by LDH assays. Solute fluxes and dual Na^+-mannitol flux analysis indicate that the resistance decrease is due to an effect of γIFN on tight junction permeability. The effects of γIFN on monolayer barrier function were not duplicated by the cytokines interleukin 1, interleukin 2, or tumor necrosis factor. We speculate that such products of activation of lymphoid cells might influence barrier function of intestinal, and perhaps other epithelia in disease states.

Introduction

Epithelial cells of many organs, including those of the intestine often intimately associate with lymphoid cells (1). The density of lymphoid cells directly underlying intestinal epithelial cells is particularly prominent in chronic disease states such as celiac sprue and idiopathic inflammatory bowel disease (2). However, it is not known if lymphoid cells can directly influence such basic physiological roles of epithelia as barrier function. When stimulated by antigens or mitogens, thymus-derived (T) lymphocytes synthesize and secrete potent mediators such as gamma-interferon (γIFN) (3–6). The biological effects of γIFN are pleiotropic but studies have largely focused on γIFN-mediated regulation of the immune response (7–10). However, many classes of cells, including epithelial cells (11) and endothelial cells (12), appear to bear surface receptors for γIFN.

In this study we assess the direct effects γIFN on an important aspect of intestinal epithelium-barrier function. For such studies, we utilize cultured monolayers of the human intestinal epithelial cell line T_{84} (13). We show that γIFN may substantially diminish intestinal epithelial barrier function as assessed in such monolayers. Moreover, γIFN appears to exert this effect by increasing the permeability of interepithelial tight junctions.

Methods

Confluent monolayers of the human intestinal epithelial cell line, T_{84}, were grown on collagen-coated permeable supports and maintained until steady state resistance to passive transepithelial ion flow was achieved as previously described (13, 14). Under these conditions, neighboring cells are adjoined by circumferential intercellular tight junctions that dramatically restrict the passive paracellular flow of ions and solutes (14). Transepithelial solute fluxes and measurement of resistance to passive ion flow were performed in modified Ussing chambers as previously described (14, 15).

Measurement of lactate dehydrogenase (LDH)[1] release was used as a means of detecting cell death. LDH content of the postexperimental supernatant from control and experimental tissues were obtained and expressed as percentage of total LDH. To determine total LDH, residual intracellular LDH levels were measured by detergent extracting cells in 1% Triton X-100 for 20 min.

For light and electron microscopy monolayers were fixed and examined as previously described (13, 14). For Nomarski imaging of unfixed, living monolayers and for fluorescent localization of the F-actin specific probe rhodamine-labeled phalloidin, monolayers were grown on glass coverslips to confluence before experimentation. These techniques were performed as previously described (14, 15).

Recombinant human γIFN was kindly provided as a > 99% pure preparation by Biogen Corp., Cambridge, MA.

Results

Baseline resistance varied from 400 to 1,200 ohm · cm^2. Such intermonolayer variation in resistance reflects intermonolayer differences in tight junction permeability (15). 72-h exposure to recombinant human γIFN at concentrations of 10–1,000 U/ml elicited progressive decreases in monolayer resistance (Fig. 1). Such large decreases in resistance were not due to gross monolayer disruption since 72 h LDH release from monolayers exposed to γIFN (1,000 U/ml) was similar to that of control monolayers without γIFN (17±3 vs. 18±4% of total, respectively, for control and γIFN exposed). Additionally, as seen in Fig. 2, Nomarski images of control and γIFN-treated

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Received for publication 3 August 1988 and in revised form 20 October 1988.

J. Clin. Invest.

0021-9738/89/02/0724/04 $2.00
Volume 83, February 1989, 724–727

1. *Abbreviations used in this paper:* LDH, lactate dehydrogenase.

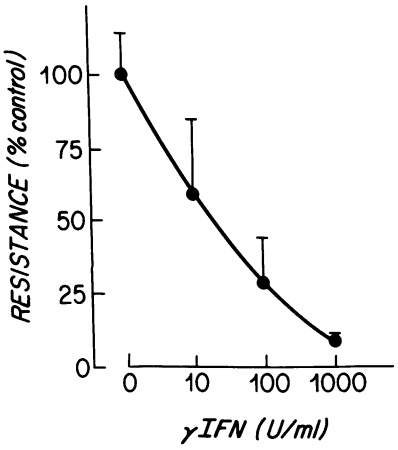

Figure 1. 72-h exposure of T_{84} monolayers to γIFN at concentrations ranging from 10 to 1,000 U/ml results in impaired monolayer resistance. Increasing concentrations of γIFN produce increasing decrements in resistance. These concentrations and exposure durations are comparable to those required for γIFN to produce its biological effects in other systems such as lymphoid tissues and endothelial cells. (*n* for each point = 4–10; Mean resistance for controls = 417 ohm × cm^2).

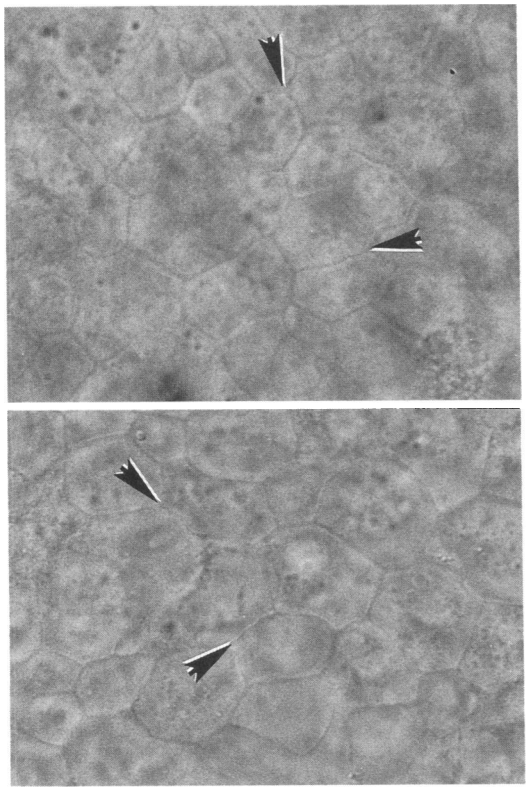

Figure 2. Nomarski photomicrographs of unfixed, living monolayers of T_{84} cells exposed for 72 h to either γIFN 1,000 U/ml (*below*) or vehicle alone (*above*). After both conditions, monolayers remain confluent and the abutment of individual cells with their neighbors can be seen as a honeycomb of subtle ridges (*arrowheads*). Magnification 1,000. Preparations were examined en face using Nomarski optics, which permits one to focus up and down through the monolayer at discrete focal planes ("optical sectioning") (23). Photographs were taken at the level of the apical membrane since this is where the intercellular tight junction, which is the rate limiting barrier to solute permeation around cells, resides. This method allows one to determine if morphologically detectable separations have occurred between cells.

monolayers did not show grossly disrupted monolayers. The plane of focus in these en face images is at the level of the apical membrane, where cells abut and form intercellular junctional contacts seen in the figure as ridges. Using this technique one can determine whether cells are grossly pulled apart (by distances of 0.5 μm and above). Thus the fall in resistance not only is not due to cell death but cannot be explained by cells pulling away from their neighbors. Monolayer barrier function was not altered by endotoxin in these experiments since endotoxin has no effect on T_{84} monolayer barrier function (not shown) and since maximal endotoxin contamination was < 0.64 ng/ml (by limulus lysate assay, courtesy of Dr. Chris Liu). Exposure to 1,000 U/ml IFN for 72 h dramatically increased the unidirectional flux (13) of the extracellular solute mannitol ($P < 0.001$) indicating an effect of γIFN on tight junction permeability (Table I). In order to determine if the γIFN elicited increase in ion permeability was attributable entirely to an effect on tight junction permeability, unidirectional dual flux analysis using ^{22}Na and ^{3}H mannitol (15, 16) was performed during the evolution of the γIFN-induced resistance change. Regression analysis of these data yielded (Fig. 3) a slope of 54 ($r = 0.96$), which is comparable to that predicted for a purely tight junctional effect on permeability under these experimental conditions (predicted = 54) (15, 16). This approach uses the incremental increase in mannitol flux, seen as the γIFN effect evolves, as a marker of the increment in paracellular permeability (15, 16). The enhancement of tight junction permeability following γIFN exposure extended to larger solutes as well (Table I: inulin, Stokes radius 11.5 Å). This IFN effect did not appear to be reversible as judged by washout experiments.

Time course experiments showed that there was no significant effect of γIFN on baseline resistance at 24 or 48 h. However, in monolayers exposed to γIFN, 1,000 U/ml, for 48 h, and subsequently mounted in chambers that were vigorously stirred, the resistance substantially deteriorated in the absence of morphologically detectable cell detachment. In contrast, the resistance of equally perturbed control monolayers was unaffected (Fig. 4). This stirring induced decrease in resistance was not accompanied by an increase in short circuit current, was accompanied by an increase in mannitol flux (10.2±0.8 vs. 24.0±1.4 nmol h^{-1} cm^{-2} for control and IFN exposed monolayers, respectively, $P < 0.001$), and Na-mannitol dual flux analysis again indicated a paracellular effect was involved (observed slope = 53, $r = 0.998$; predicted slope if effect on Na^+ flux paracellular = 54). To obtain these latter data, fluxes were obtained as sequential 20 min periods between 5 and 85 min after mounting in the chamber.

Table I. Transepithelial Flux of Extracellular Solutes

	Mannitol	Inulin
	nmol h^{-1} cm^{-2}	*nmol h^{-1} cm^{-2}*
Control	9.1±1.3	1.4±0.2
γIFN	64.0±40.0*	7.3±2.7*

Effects on extracellular solute flux of T_{84} monolayer exposure to 1,000 U/ml γIFN. Fluxes of both solutes are increased approximately sixfold by γIFN. (*n* = 6–8 for each value.)
* Both $P < 0.001$ compared with control.

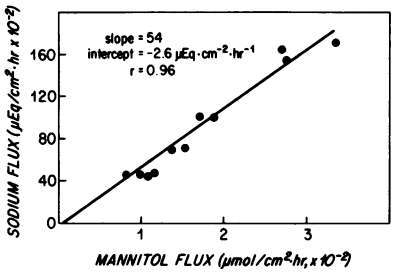

Figure 3. Dual Na^+-mannitol flux obtained from unperturbed monolayers and from monolayers exposed to γIFN for 48–56 h. Such data allow one to indirectly access whether the increment in paracellular flux, as measured by increasing mannitol-permeable pathway, fully accounts for enhanced ion flux and, therefore, decreased resistance. Analysis of such data (see text) suggest that γIFN effects of flux are wholly paracellular (i.e., transjunctional). Fluxes were obtained 5 to 25 min after mounting in the chamber. (The four control points are those with Na^+ fluxes less than 72 $\mu eq \times cm^{-2} \times h^{-1} \times 10^{-2}$.)

In contrast to γIFN, several other cytokines had no effect on monolayer resistance (Table II). In contrast to reported effects on endothelia (12), γIFN did not produce profound rearrangement of F-actin in T_{84} cells. However, subtle alterations, such as focal rearrangement of a F-actin into condensed spicules, was observed (Fig. 5). Furthermore, the general ultrastructural characteristics of T_{84} cells which we have previously described in detail (17) were unaffected by γIFN (not shown).

Discussion

The γIFN concentrations and durations of exposure which diminished epithelial barrier function, are comparable to those required to produce many of the immunoregulatory effects of γIFN (7–10) as well as the γIFN-elicited alterations in nonlymphoid cells such as endothelial cells (16). While there is no information available regarding γIFN concentrations in the subepithelial compartment in states of intestinal disease, comparable concentrations to those used here are found in the bulk fluid phase of tissue culture supernatants after activation of T lymphocytes in vitro (18). In data not shown, we found that such crude supernatants also had similar effects on monolayer barrier function to those elicited by the addition of recombi-

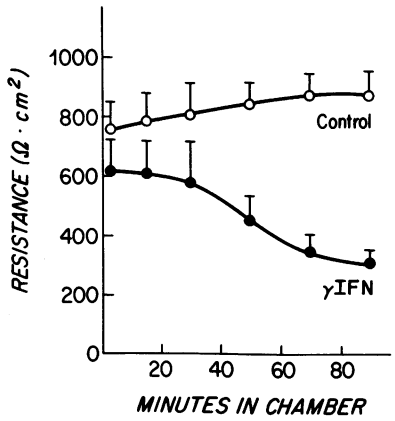

Figure 4. Time course of monolayer resistance after 48 h exposure to γIFN or vehicle (control) and subsequent mounting in modified Ussing chambers in which solutions were stirred. Under such conditions resistance declines in γIFN exposed, but not in control, monolayers. Similar decreases were not observed if buffer was unstirred. This suggests that even before the effects of γIFN on baseline resistance are seen, γIFN may increase the "fragility" of epithelial barrier function to minor physical stresses. (n = 6.)

Table II. Effects of Cytokines on Monolayer Resistance

	Resistance change as percent baseline
Control	−4±4%
γIFN (100 U/ml)	−74±6%
γIFN (200 U/ml)	−81±8%
γIFN (200 U/ml) + TNF (100 U/ml)	−72±8%
γIFN (1000 U/ml)	−96±7%
TNF (100 U/ml)	−1±2%
IL-1 (10 U/ml)	−5±3%
IL-1 (100 U/ml)	−6±5%
IL-2 (200 U/ml)	−4±3%

Effects on T_{84} monolayer resistance of 72 h exposure to various cytokines. Concentrations are selected on the basis of the upper range of concentrations at which known biological effects are studied in other systems. Only γIFN affects resistance. (n = 5–20 for each.)

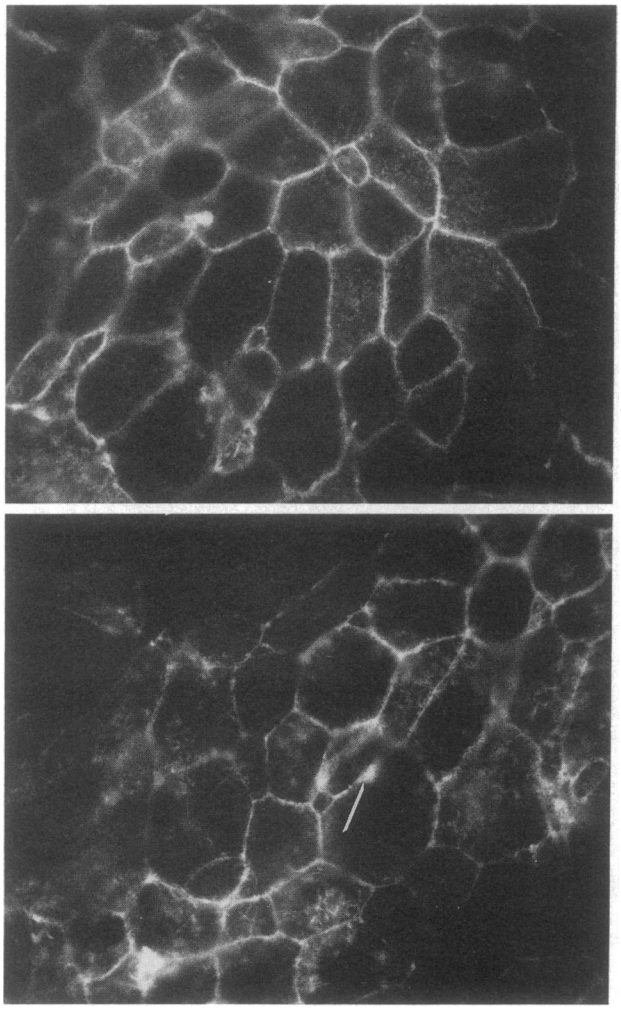

Figure 5. Fluorescent localization of f-actin, as viewed en face, in control (*top*) and γIFN (1,000 U/ml, 72 h) treated (*bottom*) monolayers. In striking contrast to endothelia (12) γIFN does not cause profound alteration in f-actin distribution in T_{84} cells. Subtle alterations, such as condensed spicules of actin (*arrowheads*) in the perijunctional actomyosin ring were noted. Although this cytoskeletal structure may be important in regulating junctional permeability (15, 18, 19) the significance of such subtle alterations is uncertain. (× approximately 1,000.)

nant γIFN alone. Such findings, in aggregate, suggest that in chronic intestinal disease states that are characterized by infiltrates of activated T lymphocytes, γIFN may in part account for diminished epithelial barrier function.

Durations of exposure to γIFN that did not affect monolayer barrier function were found to enhance the fragility of this barrier. Thus challenges normally withstood, such as enhanced reservoir turbulence, resulted in substantial loss of barrier function. Since native epithelia normally undergo mechanical stress due to peristalsis, it is possible that such γIFN exposure could be deleterious to intestinal epithelial barrier function even if the duration of exposure to this agent were < 72 h.

Lastly, since γIFN is known to affect actin distribution in some cell types (12), and cytoskeletal rearrangements are thought to influence tight junction permeability in epithelial cells (15, 19–22), we examined the effects of γIFN on f-actin distribution. Although subtle changes were detected, profound alterations such as those seen in endothelia (12), were not seen.

These data suggest that, in the intestine, γIFN may substantially affect such vital and primary physiological roles of epithelia as barrier function. It is thus possible that such effects may be partially responsible for the permeability alterations seen in chronic diseases of the intestine in which activated lymphocytes are present adjacent to the epithelium.

Acknowledgments

We thank our colleagues, especially Drs. Abul Abbas and Jordan Pober for discussions and advice. We thank Dr. Donna Wall for providing crude supernatants from activated T cells. We thank Dr. Christopher Liu for measurements of endotoxin and Susan Carlson for help in preparation and examination of monolayers.

Supported by National Institutes of Health grants DK-35932 and DK-34854.

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