Role for IL-4 in macromolecular transport across human intestinal epithelium

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Role for IL-4 in macromolecular transport across human intestinal epithelium. Am. J. Physiol. 276 (Cell Physiol. 45): 1046–C1052, 1999.—Increased epithelial permeability is associated with intestinal inflammation, but there is little information on factors that regulate barrier function in the absence of or before inflammation. We examined if interleukin (IL)-4, or serum from atopic individuals, could alter the barrier function of human colonic epithelial (T84) monolayers to antigenc-sized macromolecules. IL-4 and atopic serum significantly decreased T84 monolayer resistance and increased transepithelial horseradish peroxidase (HRP) transport. Bidirectional transport studies demonstrated that IL-4 selectively enhanced apical-to-basal movement of HRP. HRP transport induced by IL-4 was inhibited by cold (4°C) and the tyrosine kinase inhibitor genistein, but not the protein kinase C inhibitor staurosporine. Electron microscopic analysis demonstrated that both transcellular and paracellular pathways were affected. Anti-IL-4 antibodies abolished the increase in HRP transport in response to both IL-4 and serum. We speculate that enhanced production of IL-4 in allergic conditions may be a predisposing factor to inflammation by allowing uptake of luminal antigens that gain access to the mucosal immune system.

The intestinal epithelium functions as a selective barrier that facilitates nutrient absorption while limiting uptake of antigenic or noxious material from the lumen. The lamina propria immediately below the epithelial layer is densely populated with immune cells capable of responding to antigens that breach the epithelial barrier. Mediators released from activated immune cells have been shown to alter normal epithelial transport and barrier function and may possibly amplify the influx of antigenic material from the lumen. It has been well documented that inflammatory disorders of the human intestine are associated with epithelial barrier dysfunction (25, 29), but it is not clear if the epithelial perturbation is a consequence of inflammation or a predisposing factor.

Epithelial barrier dysfunction in the absence of overt inflammation has been documented in atopic patients and in animal models of allergy. Majamaa and Isolauri (20) demonstrated that small intestinal biopsy specimens from patients with atopic eczema had a greater transepithelial transport of intact proteins than biopsies from nonatopic controls. In vivo studies on patients with bronchial asthma have also indicated that intestinal epithelial permeability is elevated compared with controls (1). We recently demonstrated that allergic sensitization of rats stimulated macromolecular uptake across the intestinal epithelium (2). In those studies, basal epithelial permeability was altered in the absence of intestinal inflammation or mast cell activation.

Atopic diseases are characterized by an increase in interleukin (IL)-4-producing T lymphocytes. These can be detected in the systemic circulation (28) as well as at respiratory (4) and intestinal (12) mucosal surfaces. IL-4 functions as a switch factor for IgE synthesis from B lymphocytes (8) and stimulates the production of other T helper type 2 (Th2) cytokines (18). IL-4 has also been shown to have direct effects on human intestinal epithelium, reducing ion secretory responses, decreasing electrical resistance, and increasing permeability to small-molecular-weight probes (6, 31).

In this study, we examined the direct effect of IL-4 and serum from atopic humans on the barrier function of a model intestinal epithelium to an antigen-sized protein. Horseradish peroxidase (HRP) was chosen as the model protein for several reasons: 1) HRP can be readily measured by kinetic enzymatic assay, 2) HRP can be visualized by electron microscopy, and 3) we have previously used HRP as a sensitizing antigen in a rodent model of food allergy (2). Apical-to-basal transepithelial transport of intact HRP was used to assess the ability of T84 monolayers to restrict movement of antigenic macromolecules. We found that T84 cells grown in the presence of either IL-4 or atopic serum responded with a large increase in the transepithelial movement of HRP. IL-4-induced changes in HRP transport were maximal in the apical-to-basal direction and were mediated in part by tyrosine kinases. Electron microscopy indicated that both macromolecular transport pathways were affected; endocytic uptake of HRP was significantly increased, and tight junctions were perturbed, allowing movement of HRP through the paracellular regions between epithelial cells. These findings suggest that IL-4 production in atopic individuals may be sufficient to alter epithelial barrier function, thereby allowing an influx of antigenic material from the intestinal lumen.

METHODS

Cell culture. T84 epithelial cells were seeded (10^6 cells) on tissue culture-treated Transwell filter supports (0.4-µm pore
size, 1.2-cm² surface area; Costar, Cambridge, MA). Culture media consisted of a 1:1 mixture of DMEM and Ham's F-12 nutrient mixture (GIBCO, Grand Island, NY) supplemented with 10% (vol/vol) heat-inactivated FCS (Cansera International, Rexdale, Ontario, Canada), 1.5% HEPES (GIBCO), and 2% penicillin-streptomycin (GIBCO). Cells were grown at 37°C in 5% CO₂ for 7 days to attain polarized epithelial monolayers with transepithelial resistance (TER) measurements of >1,000 Ω·cm². Monolayer resistance was monitored throughout the experimental period using a Millicel-ERS system (Millipore, Bedford, MA). TER is a measure of the ability of T84 cells to restrict passive movement of ions.

Serum samples. Blood was obtained from atopic patients during a routine visit to the allergy clinic at McMaster Medical Centre. Patients used were age 27–62 yr (n = 4), with active atopic dermatitis, documented to have immediate skin test reactivity to multiple antigens, including ragweed and dust mites. Patients were not currently taking corticoste-roids. Serum from healthy volunteers with no history of allergy or asthma were obtained for control experiments. All serum samples were snap-frozen in liquid nitrogen and stored at −70°C before use in experiments.

Treatment of monolayers with IL-4 or serum. Confluent monolayers were cultured with 10 ng/ml recombinant human IL-4 (R & D Systems, Minneapolis, MN) added to the basal compartment (volume, 1.5 ml) of the Transwell unit. This dose was chosen based on published dose responses of the effect of IL-4 on TER (6). Atopic or control serum was added to the basal compartment at 50 µl/ml (5%), the minimum effective dose based on preliminary experiments. Cells were treated for 1, 2, or 3 days before study. In some experiments, medium containing IL-4 or serum was preabsorbed with anti-human IL-4 (1 µg/ml, R & D Systems) 2 h before addition to the epithelial monolayers.

Ussing chamber experiments. T84 monolayers on filter supports were mounted in Ussing chambers and bathed in oxygenated Krebs buffer (pH 7.35, 37°C). Krebs buffer bathing the basal surface contained (in mM) 115 NaCl, 8 KCl, 1.2 MgCl₂, 1.25 CaCl₂, 2.0 KH₂PO₄, 25 NaHCO₃, and 10 glucose as an energy source. In the apical buffer, 10 mM mannitol was substituted for glucose. After a 15-min equilibration, the potential difference across the monolayers was clamped at 1 mV (differential pulse method, 1 pulse/30 s). The resulting current deflection was measured, and TER was calculated according to Ohm's law.

 Transepithelial transport of HRP. HRP (mol wt 44,000, type VI-A, Sigma) was added to the apical or basolateral side of the monolayers at a concentration of 10⁻⁵ M, and monolayers were incubated for 2 or 18 h at 37°C. Media samples were obtained from the apical and basal compartments and assayed for HRP concentration by kinetic enzymatic assay as previously described (2). Briefly, sample was added to phosphate buffer containing H₂O₂ and o-dianisidine (Sigma). Enzyme activity was determined from the rate of increase in optical density at 460 nm. HRP transport was calculated as recovery of added HRP. In some cases, recovery under experimental conditions was expressed as percent recovery across control monolayers.

In separate experiments, cells were cooled for 1 h at 4°C before addition of HRP to the apical compartment. Cells were then incubated for 2 h at 4°C, and samples were taken from apical and basal compartments and assayed for HRP activity. Transepithelial movement of HRP was compared with cells of the same passage maintained at 37°C.

Inhibitors. T84 cells were pretreated with the tyrosine kinase inhibitor genistein (4 µM) or the protein kinase C (PKC) inhibitor staurosponine (10 nM) for 60 min before addition of IL-4 to the basal compartment. Inhibitors were added to both apical and basal compartments of the Transwells. Transepithelial transport of HRP was assessed 24 h later. Because staurosponine alone affected epithelial barrier function after incubations of >3 h, in some experiments the media were removed 2 h after IL-4 addition and replaced with fresh media for the duration of the experiment.

Electron microscopy. To examine the route of transepithelial transport of HRP, T84 monolayers to which 10⁻⁵ M HRP had been added on the luminal side were subjected to transmission electron microscopy. Briefly, 60 min after HRP addition, T84 monolayers were fixed with 2.5% glutaralde- hyde in 0.1 M sodium cacodylate buffer (pH 7.4) and then processed for 3,3′-diaminobenzidine tetrahydrochloride (DAB) cytochemistry using 0.5 mg/ml DAB and 0.01% H₂O₂ in Tris buffer. After washes in Tris-buffered saline, samples were dehydrated through a series of graded ethanol washes and embedded in Epon. Ultrathin sections were cut and placed onto mesh copper grids and stained with uranyl acetate and lead salts. An observer unaware of the treatment group (P.-C. Yang) analyzed random photomicrographs. For each experiment, total intracellular HRP was quantified by determin- ing the number of HRP-containing endosomes and total endosomal HRP area in windows (25 × 30 µm, 20/group). Tight junctions and paracellular regions were also examined for the presence of HRP.

IL-4 measurement. IL-4 concentration in serum samples was measured using a commercially available human IL-4 ELISA kit (BioSource International, Camarillo, CA). The limit of detection of the assay was >15 pg/ml.

Statistics. Data are expressed as means ± SE. Because of variability in resistance and HRP transport between monolayers from different cell passages, data were analyzed using paired nonparametric statistics (Friedman's test for repeated measures for group comparisons, Wilcoxon's signed rank test for individual comparisons); P values <0.05 were accepted as significant. In some cases, HRP transport was expressed as percent recovery across control monolayers.

RESULTS

Treatment with IL-4 or atopic serum decreases TER of T84 monolayers. TER of control monolayers increased throughout the 3-day experimental period, with a mean of 1,390 ± 50 (SE) Ω/cm² at day 1, 1,800 ± 160 at day 2, and 2,330 ± 120 at day 3. Treatment of monolayers with IL-4 resulted in a significantly lower TER (53.8 ± 3.6, 46.8 ± 3.1, and 38.7 ± 3.5% of control for days 1–3, respectively) (Fig. 1). TER was also lower after treatment with atopic serum (82.6 ± 2.1, 73.7 ± 3.8, and 68.1 ± 5.3% at days 1–3, respectively). The maximum change in TER occurred at day 3; therefore, HRP transport experiments were conducted on day 3.

Treatment with IL-4 or atopic serum increases apical-to-basal transepithelial transport of HRP. Apical-to-basal transepithelial movement of HRP over 18 h was measured 3 days after culture of the T84 monolayers with IL-4 or atopic serum. In control monolayers, intact HRP was detected at low levels in the basal compartment of the Transwells. Treatment with IL-4 increased HRP transport to 657 ± 174% of matched control monolayers. Atopic serum also increased HRP transport to 675 ± 247% of time-matched control monolayers (Fig. 2). Serum from nonatopic controls did not signifi-
IL-4 mediates transepithelial transport of HRP through tyrosine kinases. Cells were treated with genistein (4 µM) to determine the contribution of tyrosine kinases in the effect of IL-4 on transepithelial transport of HRP. Genistein had no direct effect on HRP transport in the absence of IL-4. When added to IL-4-treated monolayers, genistein inhibited the increase in HRP transport induced by IL-4, such that HRP recovery was not significantly different from the value across control monolayers (Table 1). Treatment of monolayers with the PKC inhibitor staurosporine (10 nM) alone increased the transepithelial transport of HRP, and the effects of IL-4 and staurosporine on HRP transport appeared to be additive (data not shown). Further experiments were done by pretreating T84 cells with staurosporine for 60 min and coincubating cells with staurosporine plus IL-4 for 120 min, followed by replacement with fresh media. Under these conditions, stauro-

**Fig. 1.** T84 monolayer transepithelial resistance (TER, in Ω/cm²) after treatment with 10 ng/ml interleukin (IL)-4 or 70 µl atopic serum (AS) for 1, 2, or 3 days. TER was significantly reduced after 1 day of treatment, and maximal percent change from control was observed after 3 days of treatment (n = 4–14 monolayers/group).

**Fig. 2.** Movement of horseradish peroxidase (HRP) across T84 monolayers after treatment with IL-4, atopic serum (AS), or nonatopic serum (NAS) for 3 days. HRP (10⁻⁵ M) was added to apical compartment of monolayers, and samples from apical and basal media were taken after 2 h at 37°C. HRP concentration in basal compartment was expressed as percent recovery of added HRP (n = 9–15 monolayers/group). *P < 0.05 compared with controls.

**Fig. 3.** Bidirectional HRP transport across T84 monolayers after treatment with IL-4 for 24 h. HRP (10⁻⁵ M) was added to apical compartment of monolayers, and samples from apical and basal media were taken after 2 h at 37°C. HRP concentration in basal compartment was expressed as percent recovery of added HRP (n = 9 monolayers/group). *P < 0.05, **P < 0.005 compared with control.

**Fig. 4.** Effect of temperature on transepithelial transport of HRP across T84 monolayers after treatment with IL-4 for 24 h. HRP (10⁻⁵ M) was added to apical compartment of monolayers, and samples from apical and basal media were taken after 2 h at 4°C. HRP concentration in basal compartment was expressed as percent recovery of added HRP. *P < 0.05 compared with similarly treated monolayers at 37°C. †P < 0.05 compared with control monolayers at 4°C (n = 6 monolayers/group).
Sporine did not alter the resistance or transepithelial transport of HRP, whereas IL-4 increased the transepithelial transport of HRP. Inclusion of staurosporine during the incubation with IL-4 did not affect the increase in HRP transport induced by IL-4 (Table 1).

Table 1. Effect of genistein and staurosporine on transepithelial HRP transport in the presence of IL-4

<table>
<thead>
<tr>
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<th>-IL-4 (µM)</th>
<th>+IL-4 (µM)</th>
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<tbody>
<tr>
<td>Control</td>
<td>10.1 ± 1.1</td>
<td>23.3 ± 4.0*</td>
</tr>
<tr>
<td>Genistein</td>
<td>10.7 ± 1.6</td>
<td>16.6 ± 1.7</td>
</tr>
<tr>
<td>Staurosporine</td>
<td>10.6 ± 2.2</td>
<td>21.2 ± 5.4*</td>
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Values are means ± SE of 6–9 monolayers/group and are expressed as percent recovery × 10⁻³. T84 monolayers were pretreated with genistein (4 µM) or staurosporine (10 nM) for 60 min before addition of 10 ng/ml interleukin-4 (IL-4) to basal compartment of Transwells. In experiments with staurosporine, media were removed and replaced with fresh media after 2 h. After 24 h, horseradish peroxidase (HRP) was added to apical well and monolayers were incubated for 2 h. Apical and basal media were sampled and assayed for HRP activity. *P < 0.05 compared with controls.

Sporine did not alter the resistance or transepithelial transport of HRP, whereas IL-4 increased the transepithelial transport of HRP. Inclusion of staurosporine during the incubation with IL-4 did not affect the increase in HRP transport induced by IL-4 (Table 1).

Treatment with IL-4 or atopic serum affects both transcellular and paracellular pathways. The route of apical-to-basal transepithelial HRP transport was assessed using transmission electron microscopy. Representative photomicrographs are shown in Fig. 5. Analysis of HRP within paracellular regions demonstrated that 3-day treatment with IL-4 or atopic serum induced transport through the tight junctions. Although no paracellular regions examined in control monolayers contained HRP, 70% of IL-4-treated and 45% of atopic serum-treated monolayers were positive for HRP (Table 2). Sixty minutes after addition of HRP to the luminal side of T84 monolayers, HRP was visualized within endosomal vesicles. Quantitative analysis of intracellular HRP (by measuring the area of endosomal HRP) showed that treatment with IL-4 and atopic serum significantly (P < 0.001) increased the amount of endosomal HRP compared with control monolayers. The mean area of HRP in epithelial cells increased from 2.54 ± 0.30 µm²/window in control monolayers to 8.24 ± 1.7 µm²/window in IL-4-treated monolayers and

![Fig. 5. Representative photomicrographs of T84 cells treated with media alone (A), IL-4 (B and C), or atopic serum (D) for 3 days. T84 cells were mounted in Ussing chambers and exposed to 10⁻³ M HRP for 60 min at 37°C before fixation for electron microscopy. Arrowheads indicate HRP reaction product in vesicles or in paracellular regions. Magnification: ×3,800 (A and B); ×12,000 (C), ×8,000 (D).](http://apc.all.org/)
IL-4 INCREASES TRANSEPITHELIAL MACROMOLECULE TRANSPORT

Table 2. Quantification of HRP within paracellular regions of T84 monolayers

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Regions Examined</th>
<th>HRP-Positive Regions</th>
<th>%HRP Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>65</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IL-4</td>
<td>71</td>
<td>51</td>
<td>70</td>
</tr>
<tr>
<td>AS</td>
<td>73</td>
<td>32</td>
<td>45</td>
</tr>
</tbody>
</table>

T84 monolayers were cultured for 3 days in presence of IL-4 (10 ng/ml) or atopic serum (AS, 5%) or with media alone (control). Monolayers were fixed for electron microscopy 60 min after addition of HRP to luminal side of Ussing chambers. Definitions are as in Table 1.

6.75 ± 1.3 μm²/window in atopic serum-treated monolayers (Fig. 6).

Antibodies against IL-4 inhibit the increase in transepithelial HRP transport induced by IL-4 or atopic serum. IL-4 induced a drop in TER that was inhibited by addition of anti-IL-4 antibodies at a concentration of 1 µg/ml (94 ± 6% of control resistance compared with 47 ± 6% of control with IL-4 alone). Preincubation of the IL-4 or atopic serum with anti-IL-4 antibody abolished the increase in HRP transport, such that HRP transport was not significantly different from control (151 ± 31 and 119 ± 7% of control for IL-4 and atopic serum groups, respectively) (Fig. 7). The concentration of IL-4 in the atopic serum was measured by an ELISA for human IL-4. IL-4 was below the level of detection (15 pg/ml) in all of the sera used in the experiments.

DISCUSSION

It is well established that gastrointestinal inflammation is associated with reduced barrier function (25, 29). The observation of increased intestinal epithelial permeability in a subgroup of healthy relatives of patients with Crohn’s disease (22, 24) has led to the hypothesis that increased epithelial permeability may be a predisposing factor in the development of inflammatory bowel disease. However, much of the information to date on regulation of the epithelial barrier has focused on inflammatory factors rather than possible predisposing noninflammatory factors. In this study, the effect of IL-4 and serum from atopic patients on transepithelial transport of a model protein antigen, HRP, was examined. IL-4 significantly reduced epithelial barrier function, as demonstrated by decreased resistance and increased apical-to-basal movement of HRP. Atopic serum mimicked the effects of IL-4 on HRP transport. Our studies also suggest that the effects of atopic serum were mediated by an IL-4-dependent mechanism, as shown by complete inhibition of enhanced HRP transport with anti-IL-4 antibodies. Experiments conducted at 4°C and analysis of the route of HRP transport by electron microscopy revealed that both transcellular and paracellular transport routes were affected.

IL-4 was initially described as a T-helper lymphocyte-derived growth factor for B lymphocytes (16). It has since been shown to be produced not only by T cells but also mast cells (5) and eosinophils (23). IL-4 is required for IgE production (9) and the generation of a Th2 cytokine profile (18), characterized by IL-4, IL-5, and IL-10 production. IL-4 also inhibits the production of proinflammatory mediators by macrophages (10). IL-4 is elevated in allergic disorders (28) and helminth infections (27) as well as in ileal biopsies from Crohn’s disease patients in early stages of relapse (7). IL-4 is produced by immune cells at mucosal sites in close proximity to intestinal (12) and respiratory (4) epithelia, ideally situated to modify epithelial physiology.

IL-4 clearly has effects on epithelial cells. Intestinal epithelial monolayers cultured with IL-4 have decreased TER, as well as decreased ion secretory response to secretagogues, effects that were inhibited by an antibody against the IL-4 receptor (6, 31). We have previously demonstrated enhanced transcellular antigen uptake across the intestinal epithelium in a rat model of food allergy (2), and sensitized rats are characterized by elevated IL-4 production (Berin and Perdue, unpublished observations). The current studies were designed to determine the role of IL-4 in the regulation of macromolecular transport across intestinal epithelium.
We confirmed the findings of Colgan et al. (6) by demonstrating that culture of T84 cells with IL-4 caused a significant drop in TER. TER was also monitored in monolayers cultured with atopic serum. The time frame of the drop in resistance was similar to that observed with IL-4, but the magnitude of the resistance drop was smaller. Although resistance changes suggested an alteration in tight junction permeability, it was unclear whether these changes would translate into greater permeability of a macromolecule such as HRP. Previous studies have indicated that T84 tight junctions can maintain their barrier function against macromolecules such as HRP (mol wt 44,000) even at low resistance (13). Culture of T84 cells with IL-4 for 3 days caused a large sevenfold increase in HRP transport compared with controls. In the presence of atopic serum, HRP transport was increased 10-fold over controls. Further experiments on the cellular mechanisms involved in the regulation of HRP transport by IL-4 were performed at 24 h, when significant but submaximal changes were observed. Enhanced transport of HRP across epithelial cells was maximal for the apical-to-basal direction. This result suggests that IL-4 induces uptake of luminal macromolecules rather than a nonspecific increase in permeability and implies that a significant portion of HRP transport across the cells was occurring by endocytosis. Upregulation of endocytosis in Madin-Darby canine kidney (MDCK) epithelial cells by various signals is also selective for the apical-to-basal direction (15), suggesting that the apical-to-basal and basal-to-apical transport pathways are differently regulated in epithelium.

Transepithelial HRP transport was inhibited at low temperature (4°C) under both basal and IL-4-stimulated conditions, and inhibition was of the same magnitude under both conditions. Cold acts as a general metabolic inhibitor, providing further support for endosomal transport. Our results do not rule out the possibility that cold may also alter regulation of the tight junctions. We observed that at 4°C, IL-4-treated monolayers still had an elevated transport of HRP compared with control monolayers, suggesting that a component of the increase in transport was resistant to metabolic inhibition, perhaps due to paracellular transport.

IL-4-induced changes in HRP transport were partially inhibited by genistein, suggesting the involvement of tyrosine kinases. Signaling through the IL-4 receptor is mediated through the JAK/STAT pathway, and tyrosine phosphorylation of the IL-4 receptor is a required step in the initiation of this signaling cascade (17). Therefore, it is likely that genistein was acting at the level of the initiation of the signal rather than at a more distal step. There was no effect of genistein on TER or HRP transport across control monolayers, suggesting that tyrosine kinases are not involved in the basal regulation of tight junctions or endocytic transport. Incubation of monolayers with the PKC inhibitor staurosporine during the initiation of IL-4-induced changes in epithelial barrier function had no effect on TER or HRP transport. When staurosporine was present for the duration of the experiment, it decreased basal TER and increased HRP transport, but did not affect IL-4-induced changes. This finding suggests that PKC is not involved in the initiation of signals from the IL-4 receptor.

Examination of the monolayers by electron microscopy demonstrated HRP transport via both the paracellular and transcellular routes. HRP was visualized within the tight junctions and in the paracellular regions between epithelial cells in IL-4- and atopic serum-treated monolayers but not controls. Electron microscopy also indicated that IL-4 and atopic serum enhanced transcytosis of HRP. Area of endosomal HRP transport was increased approximately threefold in IL-4- and atopic serum-treated monolayers compared with controls. This is supported by our findings that cold temperature inhibited part of the increase in HRP transport induced by IL-4 and by our findings that the direction of HRP transport appeared to be selective. There is some evidence that cytokines can regulate endocytic pathways. Tumor necrosis factor-α (TNF-α) enhances endocytosis in isolated hepatic endothelial cells (21). Interferon-γ (IFN-γ) has recently been shown to cause a greater and more rapid uptake of antigen by endocytosis in nasal epithelium (30). A wide range of signaling pathways can enhance apical-to-basal endocytosis in MDCK epithelial cells (15), suggesting that many different stimuli regulate the traffic of macromolecules across epithelial barriers. Many of the factors that enhance the endocytic pathway also increase tight junction permeability, including TNF-α (14), IFN-γ (19), and cholinergic stimuli (3). This is perhaps due to shared signaling pathways that regulate both endocytosis and tight junctions.

Patients with allergic diseases have been shown to have elevated intestinal permeability. This is observed even when the involved site is skin (20) or lung (1), indicating that a circulating factor may be responsible for the alteration in epithelial barrier function. IL-4 has been measured in serum from atopic patients, and previously reported levels range from 231 to 2,900 pg/ml in atopic patients (11). We were unable to detect IL-4 within the serum by ELISA, suggesting that if IL-4 was present it was in a very low concentration. However, preabsorption of the serum with anti-IL-4 antibody completely abolished the effect of atopic serum on HRP transport, indicating that IL-4 was an active factor in the serum. It is difficult to reconcile the complete inhibition of the effects of atopic serum by anti-IL-4 antibodies with our inability to detect IL-4 within the serum, since previously published dose-response curves with IL-4 indicate that picogram levels of IL-4 are insufficient to alter TER (6). However, it is possible that other factors present within serum act as either “priming” agents or in a synergistic fashion to enable very small doses of IL-4 to alter epithelial barrier function. Studies in progress implicate synergistic factors in the permeability changes.

Allergic diseases and inflammatory disorders are associated with an increase in intestinal epithelial permeability. IL-4 production is elevated not only in allergy, but also in intestinal mucosa in early stages of...
relapse in Crohn’s disease (7). We have demonstrated that IL-4 compromises epithelial barrier function to antigens by increasing both paracellular permeability and transcellular uptake. Therefore, IL-4 may play an important role in the pathophysiology of allergic reactions or mucosal inflammation by enhancing the uptake of antigens or bacterial products from the gastrointestinal lumen.

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