Epithelial Ion Transport and Barrier Abnormalities Evoked by Superantigen-Activated Immune Cells Are Inhibited by Interleukin-10 but Not Interleukin-41

JUN LU, DANA J. PHILPOTT, PAUL R. SAUNDERS, MARY H. PERDUE, PING-CHANG YANG and DEREK M. MCKAY2

Intestinal Disease Research Programme, McMaster University, Hamilton, Ontario, Canada
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ABSTRACT
Many studies have indicated an association between bacteria and the severity of enteric secretory or inflammatory disorders. We previously showed that monolayers of human T84 epithelial cells display altered ion transport and permeability after coculture with Staphylococcus aureus enterotoxin B (SEB, a model superantigen)-activated immune cells, where interferon-γ and tumor necrosis factor-α were key mediators in the pathophysiology. Here we examined whether the regulatory T helper type 2 cytokines, interleukin (IL)-10 and IL-4, could prevent these epithelial irregularities. T84 monolayers were cocultured with human peripheral blood mononuclear cells (PBMC) or T cell-enriched, monocyte-depleted PBMC (T + B cells) ± SEB for 20 hr in the presence or absence of IL-10 or IL-4. Subsequently, T84 monolayers were mounted in Ussing chambers and ion transport (short-circuit current (Isc) and ∆Isc evoked by forskolin) and permeability (ion resistance and probe fluxes) were assessed. IL-10 dose-dependently inhibited the increased T84 permeability and the reduced responsiveness to forskolin that were evoked by coculture with SEB-activated PBMC or T + B cells. Similar changes in T84 function occurred in response to conditioned medium from SEB-activated immune cells; however, addition of IL-10 to the conditioned medium did not prevent the changes in epithelial function. In contrast, when PBMC were stimulated with SEB in the presence of IL-10, the subsequent conditioned medium was less effective in evoking altered epithelial function. These data suggest that the effect of IL-10 was due to effects on the immune cells and not directly on the epithelium. In contrast to IL-10, IL-4 did not ameliorate any of the immune-mediated changes in T84 function. We conclude that IL-10 can reduce the epithelial functional changes caused by SEB-activated immune cells and this data adds further support for IL-10 immunotherapy in the treatment of intestinal secretory or inflammatory disorders.

Bacterial SAgs are potent stimuli of immune activity, activating T cells by cross-linking major histocompatibility class II antigens (MHC II) on antigen-presenting cells with an outside domain of the Vβ chain of the TcR (Webb and Gascoigne, 1994). By bypassing the antigen-specific cleft of the TcR, a single SAg can polyclonally stimulate ≤25% of T cells. This ability of SAgs to activate T cells, both CD4+ and CD8+ cells (Herrmann et al., 1992), via the Vβ chain of the TcR has been used to implicate SAgs of unknown identity in inflammatory diseases such as rheumatoid arthritis (Paliard et al., 1991), diabetes (Conrad et al., 1994) and multisystem vasculitis (Kawasaki disease) (Curtis et al., 1995); altered Vβ TcR expression has been recorded in these conditions. Recently, a role for SAgs in idiopathic bowel disorders has been postulated (Ibbotson and Lowes, 1995). For instance, some patients with Crohn’s disease can have increased numbers of circulating and mesenteric lymph node Vβ8+ T cells (Posnett et al., 1990), and peripheral blood T cells from patients with Crohn’s disease can have altered responsiveness to in vitro stimulation with bacterial SAgs (Baca-Estrada et al., 1995). Given the increasing apparent connection between the intestinal microflora, aberrant immune cell activity and gut disturbances (Sartor, 1997) we initiated studies to assess the ability of bacterial SAgs to modulate enteric epithelial function.

Many enteropathies, including IBD, are associated with altered epithelial function such as increased permeability.

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2 Derek M. McKay, Ph.D., Intestinal Disease Research Programme, HSC–3N5, Department of Pathology, McMaster University, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5. E-mail: mckayd@fhs.mcmaster.ca

ABBREVIATIONS: CD, cluster designation; EPEC, enteropathogenic Escherichia coli; HRP, horseradish peroxidase; IFN–γ, interferon-γ; IL, interleukin; Isc, short-circuit current; MHC II, major histocompatibility class II antigens; PBMC, peripheral blood mononuclear cells; SAgs, superantigens; SEB, Staphylococcus aureus enterotoxin B; T + B, T and B cells (monocyte-depleted); TcR, T cell receptor; T11 or T12, T helper cells types 1 and 2; TNF–α, tumor necrosis factor-α; Vβ, variable portion of the β chain of the T cell receptor.
and electrolyte secretion (that creates driving forces for water movement) and diminished responses to known secretagogues (Kachar et al., 1995). We have reported that monolayers of the human colonic T84 epithelial cell line (a model Cl–-secretory epithelium; Barrett, 1993) display altered barrier and ion transport functions when cocultured with SEB-activated PBMC (McKay and Singh, 1997). IFN-γ and TNF-α were implicated as key mediators in these epithelial irregularities, suggesting a T helper 1 (Th1)-dominated event. In further exploring the mechanism of these SEB-induced changes in epithelial physiology, we hypothesized that the Th2-type cytokines, IL-10 and IL-4 (Banchereau and Rybak, 1994; Mosmann, 1994), could prevent some, or all, of the epithelial irregularities. We have also shown: 1) that PBMC in which the T cells have been specifically activated through the TcR with an anti-CD3 directed antibody, significantly alter the ion transport and permeability characteristics of T84 monolayers (McKay et al., 1996b); and 2) T84 monolayer permeability is increased by enteropathogenic E. coli infection (Philpott et al., 1996). Consequently, we also examined the ability of IL-10 to modulate the epithelial dysfunction evoked by these latter two treatments for comparison with SEB-induced epithelial functional changes.

Our data illustrate that IL-10 (by affecting the immune cells and not the epithelium directly), but not IL-4, significantly reduces the alterations in epithelial physiology evoked by coculture with SEB-, or anti-CD3-activated immune cells, but was ineffective in ameliorating an E. coli-induced increase in epithelial permeability. These findings support the contention that IL-10 immunotherapy could be beneficial for a cohort of patients with abnormal intestinal epithelial function. We add the cautionary note that the effectiveness of such therapy may hinge on the recognition of the mechanism responsible for the altered gut function, for example, immune dysregulation versus bacterial infection.

**Materials and Methods**

**Epithelial Cells**

The human T84 crypt-like colonic epithelial cell line was chosen as a model epithelium (Barrett, 1993). As previously described (McKay et al., 1996b) T84 cells were maintained and passaged in a 1:1 mixture of Dulbecco’s modified Eagle medium and Ham’s F-12 medium supplemented with 10% (vol:vol) fetal calf serum, 1.5% (vol:vol) HEPES and 2% (vol:vol) penicillin-streptomycin (all from Gibco Laboratories, Grand Island, NY). T84 cell monolayers (passages 55–70) were grown on tissue culture-treated semipermeable filter supports (0.4-μm pore size, 1-cm² surface area; Costar Inc., Cambridge, MA) under standard conditions for 7 to 10 days at 37°C. Monolayers with transepithelial resistance values of ≥1000 Ω/cm² were used in this study.

**Immune Cells**

Blood samples were collected from healthy volunteers (male and female; age range 20–40; volunteers with allergies or on medication were excluded) into heparinized tubes and then fractionated in to one of three immune cell populations. (1) PBMC: were isolated from theuffy coat interface after ficoll density centrifugation. These cells are a mixture of T cells, B cells and monocytes (McKay et al., 1996b). (2) T + B cells: PBMC were plated onto sterile 10-cm-diameter Petri dishes and incubated at 37°C for 2 hr to allow monocyte adherence to the plastic and the nonadherent immune cells were obtained. This immune cell population was ~85% CD3+ T cells, with the remainder being predominantly B cells as judged by size and granularity on fluorescent activated cell sorting analysis (~2% CD14+ cells); for convenience this population of cells has been designated T + B cells. (3) T cells: the T + B cell population was further puriﬁed using MACs magnetic cell sorting (Miltenyi Biotec Inc., Auburn, California, USA). Lymphocytes (10⁶) were incubated with 10 μl of colloidal super-paramagnetic microbeads conjugated to mouse anti-human CD3 antibodies (Miltenyi Biotec Inc.) for 15 min at 4°C and the cell suspension was then passed through an LS separation column mounted in a Midi-MACS magnet. After washing with buffer (PBS, 2 mM EDTA, 0.5% w/v bovine serum albumin), the column was removed from the magnet and the CD3+ T cells eluted with cold buffer. All immune cell preparations were resuspended in culture medium at 10⁵/ml and were used within 30 min of isolation.

**Immune Cell Activation: Cytokine Production**

Immune cells (10⁶ cells) were exposed to 1 μg of SEB (Sigma Chemical Co., St. Louis, MO; endotoxin-free) and cell-free supernatants were collected 20 hr later, frozen and stored at −70°C. The levels of IL-2 (Advanced Magnetics, Cambridge, MA) as a specific indicator of T cell activation, TNF-α (Biotrack, Oakville, Ontario, Canada) and IFN-γ in conditioned medium from PBMC and T + B cells were measured by enzyme-linked immunosorbent assay (ELISA) and were determined by sandwich ELISA. All assays were conducted in duplicate serial dilutions. We examined TNF-α and IFN-γ levels since our previous studies implicated these cytokines in the pathophysiology evoked by SEB-activated PBMC (McKay and Singh, 1997).

**Coculture Studies**

**Immune cells stimulated by SEB.** Confluent T84 monolayers were cocultured with SEB (1 μg/well)-activated immune cells ± human recombinant IL-10 (1, 5, 10 or 20 ng/ml; R & D Systems Inc., Minneapolis, MN) for 20 hr. The immune cells, SEB and IL-10 were all added simultaneously to the basal compartment of the coculture well (McKay et al., 1996a). In some experiments, the T + B cells were preincubated with IL-10 (10 ng/ml) for 24 hr before initiation of the coculture, while in others the IL-10 was added 2 hr after the SEB-T + B cell-epithelial cocultures had been established. Another series of experiments was conducted in which human recombinant IL-4 (R & D Systems Inc.) was substituted for IL-10. IL-4 was used at 10 ng/ml for direct comparison with the IL-10 experiments. Controls consisted of T84 monolayers cultured: a) in medium only, b) exposed to IL-10 or IL-4 only for 20 hr, c) cocultured with nonactivated immune cells and d) cocultured with activated immune cells in the absence of IL-10 or IL-4.

Additional experiments were conducted to define the cellular target for the IL-10. PBMC or T + B cells were exposed to SEB (1 μg/10⁶ cells), in the absence of T84 cells, and the cell-free conditioned medium collected 20 hr later. This conditioned medium was diluted 1:1 (i.e., 50% concentration) in fresh medium and was then added, ± IL-10 (10 ng/ml), to the basal compartment of culture wells containing naive confluent T84 monolayers. Epithelial permeability and ion transport characteristics were assessed in Ussing chambers 20 hr later. The reciprocal approach was also used, where PBMC were activated with SEB in the presence of IL-10, and the resultant conditioned medium collected and used in culture studies.

**Immune cells stimulated by anti-CD3 antibodies.** For comparison with SEB-induced epithelial dysfunction, experiments were performed in which T84 monolayers were cocultured with PBMC activated by immobilized antibodies to the CD3 component of the T cell receptor (anti-CD3 at 1 μg/well; OKT3, Coulter Immunology, Miami, FL). ± IL-10 (10 ng/ml). We have previously shown that the presence of monocytes is critical for the anti-CD3-induced epithelial pathophysioloign (McKay et al., 1996b) and therefore only PBMC were utilized.

**EPEC.** EPEC infection causes increased T84 permeability (Philpott et al., 1996). We examined the ability of IL-10 to modulate...
increased epithelial permeability due to EPEC infection for comparison with immune-mediated changes in epithelial physiology. Following a published protocol (Philpott et al., 1996b), confluent T84 monolayers were apically infected with \(5 \times 10^7\) EPEC (strain E2348, serotype O127:H6) colony forming units \(\pm \) IL-10 (10 ng/ml, added to the basal compartment of the culture well). Transepithelial ion resistance was measured before and 18 hr after infection.

**Epithelial Function: Ussing Chamber Studies**

Detailed methodology has been reported (McKay et al., 1996b) and is given here only in brief.

**Ion transport.** Following coculture, T84 monolayers were mounted in specialized Ussing chambers (Precision Instrument Design, Tahoe City, CA) and bathed in normal, aerated Krebs solution (containing either 10 mM glucose or mannitol) and maintained at 37°C by a surrounding heated water jacket. The spontaneous potential difference across the monolayer was maintained at 0 V by a voltage-clamp apparatus (WPI Instruments, Narco Scientific Missisauga, Ontario, Canada) and the injected short-circuit current (Isc, \(\mu A/cm^2\)) continuously monitored as an indicator of net active ion transport. Baseline Isc was recorded after a 15 min equilibration period. Evoked changes in Isc (\(\Delta I_{sc}\)) were elicited by the addition of forskolin (10 \(\mu M\); Sigma Chemical Co.) to the basolateral surface of the T84 monolayer and the maximum change in Isc was recorded. Forskolin directly activates adenylate cyclase and causes Cl⁻ secretion via the elevation of intracellular cAMP (Barrett, 1993).

**Permeability.** The epithelial barrier to the passive movement of ions was assessed using the differential pulse method to measure transepithelial ion resistance. T84 monolayers were periodically clamped to 1 mV, the resulting current spikes were measured and resistance (in \(\Omega/cm^2\)) was then calculated according to Ohm’s law. Luminal-to-serosal fluxes of \(^{51}\text{Cr}-\text{EDTA}\) (~360 Da; Radiopharmacy, McMaster-Chedoke Hospital, Hamilton, Ontario, Canada) and the protein antigen, horseradish peroxidase (HRP; 44 kDa; Sigma Chemical Co.) were conducted. \(^{51}\text{Cr}-\text{EDTA}\) at 2.4 \(\mu\text{Ci}/\text{ml}\) (8.5 \(\mu\text{M} \text{EDTA}\)) was added to the luminal side of Ussing-chambered T84 monolayers and osmotically balanced by the addition of nonradioactive Cr-EDTA to the serosal side of the chamber. After a 40 min equilibration period, a “hot” sample (50 \(\mu l\)) was obtained for determination of probe specific activity and 1 ml samples were taken from the serosal (“cold”) buffer at 30 min intervals over a 1 hr period and replaced with the appropriate nonradioactive buffer. Radioactivity was determined in a \(\gamma\)-counter and flux rates calculated as nmol/hr/cm² (McKay et al., 1996b).

To determine the transepithelial movement of HRP, T84 monolayers were exposed to T + B cells \(\pm\) SEB for 24 hr, then removed from their coculture wells and rinsed in fresh media. The T84 monolayers were then placed in new sterile culture wells with fresh media and HRP (10⁻⁵ M) was added to the apical side of the monolayer. Samples of medium (1 ml) were removed from the basal compartment 24 hr later and HRP was determined by my assaying enzyme activity (Maehly and Chance, 1954). A 150 \(\mu l\) aliquot of sample was added to 800 \(\mu l\) of phosphate buffer containing 0.003% (v/v) of \(\text{H}_2\text{O}_2\) and 80 \(\mu g/ml\) of o-diamidine (both Sigma Chemical Co.). Enzyme activity was then determined from the rate of increase in optical density at 480 nm over a 2 min period (Berin et al., 1997). The amount of HRP recovered in the basal compartment of the culture well is expressed as the percentage of total HRP activity added to the apical side of the monolayer.

**Epithelial Integrity**

We have previously shown that epithelial cytotoxicity was unlikely to be a major contributing factor to the changes in epithelial physiology (McKay and Singh, 1997). Here we examined epithelial ultrastructure using transmission electron microscopy. Following coculture, T84 monolayers were fixed in 2% gluteraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 2 hr at 4°C and then trans-ferred to 0.1% M cacodylate buffer for 24 hr at 4°C. The preparations were then postfixed in 1% osmium tetroxide and incubated in saturated uranyl acetate (2% v/w) before being dehydrated and cleared in propylene oxide and embedded in Epon. Ultrathin sections (60 nm) from 2 monolayers/experimental condition were stained with 0.4% lead citrate and randomly selected sections, on coded slides, were examined by a single investigator (P-C.Y.) on a Joel, JEM-1200 EX electron microscope.

**Analyses**

All data are given as the mean ± standard error of the mean (S.E.M.) and unless stated otherwise, the n values refer to the number of experiments (i.e., number of blood donors) in which 2 to 4 epithelial monolayers were examined per condition. Due to variability between epithelial passages (noted by other investigators also (Adams et al., 1993)), some data have been normalized to time-matched control monolayers and are presented as percentage of control. Data were analyzed using one-way analysis of variance (ANOVA) followed by post-hoc comparisons with the Newman-Keuls test and a level of statistically significant difference accepted at P < .05.

**Results**

SEB evokes immune cell cytokine production. Cytokine production was used as an indication of immune cell activation. As shown in figure 1, SEB activation of PBMC or T cell-enriched, monocyte-depleted (T + B cells) cells resulted in a dramatic increase in the T cell-specific cytokine IL-2, as well as IFN-γ and TNF-α. The SEB-elicted increase in these 3 cytokines was significantly reduced in the presence IL-10 (10 ng/ml) (fig. 1). In contrast, the synthesis of IFN-γ and TNF-α by SEB-activated PBMC was not affected by cotreatment with IL-4 (10 ng/ml): IFN-γ = 0.2 ± 0.1, 8.6 ± 3.7, 9.4 ± 3.9 ng/ml and TNF-α = 0, 17.8 ± 5.0, 29.3 ± 5.3 ng/ml for nonactivated PBMC, SEB-activated PBMC and SEB-activated PBMC + IL-4, respectively (n = 3; mean ± S.E.M.).

SEB-activated immune cells evoke changes in epithelial permeability and ion transport. Naïve T84 monolayers treated with SEB only showed no significant alterations in their ion transport or permeability characteristics (fig. 2) (McKay and Singh, 1997).

Baseline Isc was not consistently altered under any of the experimental conditions used throughout this study. T84 cells cocultured with nonactivated PBMC, T + B cells or purified T cells for 20 hr displayed transepithelial resistances and secretory responses to forskolin that were not significantly different from epithelial monolayers cultured in growth medium only (fig. 2). In contrast, after coculture with SEB-activated PBMC or T + B cells epithelial secretory responses to forskolin were reduced to 23% and 46% of control values, respectively (fig. 2). Also, coculture with SEB-activated PBMC and T + B cells for 20 hr resulted in ~60% reduction in transepithelial resistance. The drop in T84 resistance caused by coculture with SEB-activated T + B cells was paralleled by increased transepithelial flux of \(^{51}\text{Cr}-\text{EDTA}\). Similarly, T84 monolayers had reduced barrier function to the passage of HRP over a 24 hr period after the exposure to SEB-activated T + B cells (table 1). Exposure of T84 monolayers to purified T cell preparations (>95% CD3⁺) ± SEB for 20 hr, did not result in any consistent change in epithelial ion transport or permeability (fig. 2).

IL-10 reduces T84 dysfunction elicited by coculture with SEB-activated immune cells. Addition of 10 ng/ml of
IL-10 to T84 monolayers for 20 hr did not alter epithelial permeability (1807 ± 356 and 1672 ± 211 Ω/cm² for controls and T84s exposed to IL-10, respectively) or responsiveness to forskolin (71.5 ± 6.8 and 72.5 ± 4.8 μA/cm² respectively; 4–5 monolayers/condition). These findings show that IL-10 had no direct acute effects on T84 Isc or permeability.

The addition of IL-10 to cocultures of T84 monolayers and SEB-activated T cells dose-dependently prevented the epithelial barrier and ion transport abnormalities (fig. 3). For example, IL-10 (10 ng/ml) added to the coculture well re-established transepithelial resistance and ΔIsc to forskolin to 65% and 70% of control values, respectively compared to 40% and 45% of control values in the absence of IL-10. Similarly, IL-10 significantly ablated the increased permeability to larger molecules that occurred in the presence of SEB-activated T + B cells (table 1). Pretreatment of the T + B cells with IL-10 for 20 hr before their use in coculture studies also resulted in a significant reduction in the epithelial abnormalities due to coculture with SEB-activated T + B cells (table 2), however, this was not significantly different compared to when IL-10 was added at the beginning of the coculture (fig. 3). Moreover, when T84 and SEB-activated T + B cells cocultures were established 2 hr before the addition of IL-10, no beneficial effect of IL-10 treatment was observed (table 2). IL-10 treatment also partially prevented the epithelial abnormalities evoked by a 20 hr coculture with SEB-activated PBMC (fig. 4).

IL-10 reduced the changes elicited in T84 monolayers by coculture with PBMC, in which T cell activation had been initiated by immobilized anti-CD3 antibodies: 1) transepithelial resistance: control T84 monolayers = 1871 ± 231, T84 + PBMC + anti-CD3 = 759 ± 176, T84 + PBMC + anti-CD3 + IL-10 = 1132 ± 247 Ω/cm²; 2) ΔIsc to forskolin: control T84 monolayers = 75.1 ± 4.8, T84 + PBMC + anti-CD3 = 31.7 ± 6.8, T84 + PBMC + anti-CD3 = 49.2 ± 8.4 μA/cm² (p and #P < .05 compared to other groups, mean ± S.E.M., n = 6).

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**TABLE 1**

Epithelial (T84) permeability is increased by SEB-activated T + B cells

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Medium only</th>
<th>T + B cells + SEB</th>
<th>T + B cells + SEB + IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>51Cr-EDTA flux (nmol/h/cm²)</strong></td>
<td>2.6 ± 0.7</td>
<td>8.5 ± 2.2*</td>
<td>3.4 ± 1.0</td>
</tr>
<tr>
<td>HRP transepithelial movement (% of total added)</td>
<td>0.0024 ± 0.0005</td>
<td>0.0285 ± 0.0117*</td>
<td>0.0062 ± 0.0022</td>
</tr>
</tbody>
</table>

* P < .05 compared with medium control; n = 4–6 T84 monolayers/condition; mean ± S.E.M.

**Discussion**

Inappropriate immune responses and the gut microflora are being increasingly implicated in the pathophysiology of enteric disease (Sartor, 1997). Bacterial SAGs are potent T cell stimuli and a number of studies have provided evidence...
suggested that SAgs may be involved in intestinal disease (Ibbotson and Lowes, 1995). However, while the immunomodulatory effects of SAgs continue to be the focus of extensive research efforts, there is a paucity of data on how bacterial SAgs may directly or indirectly influence intestinal epithelial function. In assessing the impact of SAgs on epithelial function, we have previously documented that SEB (a model SAg)-activated PBMC evoked significant changes in epithelial (T84 monolayers) permeability and ion transport. These epithelial abnormalities were abrogated, at least partially, by neutralization of IFN-γ and TNF-α and were not due to decreased epithelial viability (McKay and Singh, 1997). The latter finding is complemented by the current epithelial ultrastructural observations, that revealed no major differences between control and treated T84 monolayers. Moreover, given the role of IFN-γ and TNF-α in this in vitro model of SEB-induced immune-mediated epithelial dysfunction, we reasoned that Th2-type cytokines might prove beneficial in preventing some, or all, of the epithelial irregularities.

Interleukin 10 is an anti-inflammatory cytokine that can inhibit the activity of macrophages, natural killer cells and Th1 cells. In the context of enteric inflammation, pro-inflammatory cytokine (IL-1β, TNF-α) production by mitogen-stimulated immune cells from patients with inflammatory bowel disease is reduced in the presence of IL-10 and enema formulations of IL-10 have been shown to be beneficial in the treatment of some patients with Crohn’s disease (Schreiber et al., 1995; van Deventer et al., 1997). However, the ability of IL-10 to prevent immune-mediated changes in epithelial function that are often characteristic of enteric disease is not well defined.

The present study confirms our earlier findings that SEB-induced immune activation results in distinct changes in epithelial transport (reduced responsiveness to cAMP-mediated Cl− secretory stimuli) and barrier (increased permeability) functions (this is likely to be a general consequence of exposure to SAgs as we have preliminary data illustrating that the novel SAg, Yersinia pseudotuberculosis mitogen, evokes virtually identical epithelial abnormalities as SEB (McKay et al., 1998)). Increased flux of 51Cr-EDTA and HPR are generally considered indicative of increased paracellular and transcellular permeability, respectively. We have not conducted studies to determine whether the increased transcellular permeation of the large antigenic HPR was via the transcellular route (due to nonspecific endocytosis) or the paracellular shunt pathway. However, independent of the route of movement, exposure to SEB-activated immune cells elicits a significant defect in epithelial barrier function (at least to molecules of ~40,000Da). The in vivo implication of this would be increased movement of antigenic substances from the gut lumen into the mucosa which could evoke the initiation of an immune/inflammatory cascade. In extending these observations we demonstrate here that IL-10 partially and dose-dependently prevented the SEB-immune mediated pathophysiology. This propitious effect of IL-10 was apparent when SEB was used to activate PBMC or T + B cells. We

| Table 2: Effect of IL-10 treatment regimes on SEB + T + B cell-mediated epithelial dysfunction |

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Media only</th>
<th>T + B cells + SEB</th>
<th>T + B cells + SEB + IL-10 (10 ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance (Ω/cm²)</td>
<td>2287 ± 330</td>
<td>784 ± 236&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1711 ± 290&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ΔIsc to forskolin (µA/cm²)</td>
<td>52.8 ± 3.0</td>
<td>24.3 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.3 ± 1.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-10 (10 ng/ml) added 2 hr after the initiation of the coculture</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Resistance (Ω/cm²)</td>
<td>1957 ± 385</td>
<td>639 ± 120&lt;sup&gt;a&lt;/sup&gt;</td>
<td>776 ± 168&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ΔIsc to forskolin (µA/cm²)</td>
<td>67.7 ± 3.7</td>
<td>25.7 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31 ± 3.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a,b</sup> P < .05 compared with medium control and to designate statistically different groups; n = 2–3 experiments with 2–4 T84 monolayers/condition; mean ± S.E.M.
draw attention to the fact that circulating immune cells are not identical to resident mucosal cells (although peripheral cells are recruited to the site of insult and may be more active that resident cells). However, SEB has been found to activate human lamina propria and intraepithelial cells (Sperber et al., 1995) and we have preliminary data indicating that SEB-activated muscosally-derived immune cells can evoke changes in T84 function that are similar to those observed with PBMC (unpublished observation). The recognition that IL-10 can prevent epithelial functional changes evoked by exposure to SEB-activated T cells is in accordance with in vivo studies where SEB-induced lethal endotoxemia is mediated by TNF-α and is antagonized by IL-10 (Florquin et al., 1994; Howard et al., 1993). It is also noteworthy that SEB-activated purified T cells (>95% CD3⁺) did not affect epithelial function and this most likely reflects the absence of antigen-presenting cells expressing MHC II, and other accessory molecules (e.g. CD28) that are required for efficient T cell activation by SAgs (Fraser et al., 1992).

The beneficial effect of IL-10 was only observed when the cytokine was added before, or at the beginning of the coculture and was ineffective when added after the epithelial-SEB-immune cell cocultures had been established. In comparison, the ability of IL-10 to prevent SEB-induced lethal shock in mice (Bean et al., 1993) or IFN-γ-induced increases in epithelial permeability (Madsen et al., 1997) were optimal when IL-10 was administered before the experimental treatment. Additionally the spontaneous inflammation that occurs in IL-10-deficient mice can be significantly reduced by in vivo administration of IL-10, but only if this treatment occurs before the inflammation is manifest (Berg et al., 1996). These studies illustrate the prophylactic properties of IL-10 and further investigation is needed to define optimal treatment strategies for established disease.

Preliminary observations have been presented indicating the presence of an IL-10 receptor on murine and human epithelial cells (Panja et al., 1996). To identify the cellular

![Fig. 5. Bar graphs showing that exposure to conditioned medium (CM) from SEB-activated peripheral blood mononuclear cells (A-PBM) or activated-T + B cells for 20 hr results in reduced T84 monolayer ion resistance and secretory responsiveness to forskolin (10⁻⁵ M). These events were not significantly affected by the addition of IL-10 (10 ng/ml) to the conditioned medium (+, P < .05 compared to controls (Con); n = 3 experiments with 2–4 monolayers/condition; T + B cells, T cell enriched, monocyte depleted peripheral blood lymphocytes; mean ± S.E.M.).

<table>
<thead>
<tr>
<th>Conditioned medium made in the presence of IL-10 evokes less epithelial dysfunction</th>
<th>% of Control response</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC-SEB CM (50%)</td>
<td>PBMC-SEB CM (50%) made in the presence of IL-10*</td>
</tr>
<tr>
<td>Transepithelial resistance</td>
<td>46.9 ± 3.2b</td>
</tr>
<tr>
<td>∆Isc to forskolin</td>
<td>35.8 ± 3.8b</td>
</tr>
</tbody>
</table>

CM, conditioned media from PBMC (10⁶/ml) exposed to SEB (1 µg/ml) for 24 hr.
* IL-10 (10 ng/ml) was added to PBMC+SEB cultures and CM collected 24 hr later. ** P < .05 compared with control and other group; n = 8 T84 monolayers; mean ± S.E.M.
target for IL-10, we assessed the ability of IL-10 to prevent the epithelial dysfunction evoked by conditioned medium from SEB-activated immune cells and tested the effectiveness of conditioned medium made in the presence of exogenous IL-10. IL-10, at a concentration effective in modulating the functions of mature T cells in cell culture (20 hr) with SEB-activated peripheral blood mononuclear cells (A-PBM) or activated T + B cells (T + B, T cell enriched, monocyte depleted peripheral blood lymphocytes; *#, P < .05 compared to controls (Con); P < .05 compared to other groups; n = 2–5 experiments with 2–4 monolayers/condition; mean ± S.E.M.).

In conclusion, we found that IL-10 did not prevent SEB-induced epithelial abnormalities caused by immune activation by bacterial (SEB) and immunological (anti-CD3) stimuli, we compared this with any putative ability to abrogate epithelial functional changes as a consequence of bacterial infection. In contrast to immune-mediated changes, IL-10 did not affect the increase in epithelial permeability caused EPEC infection. These data again testify that IL-10 has no immediate direct effects on epithelial barrier function. However, this does not negate the possible therapeutic benefit of IL-10 in the treatment of a variety of T-cell mediated enteropathies (e.g. such as graft-versus-host disease) and transplant rejection (MacDonald and Spencer, 1992).

Interleukin 4 may have therapeutic applications. For example, IL-4 can inhibit IFN-γ production by human PBMC (Peleman et al., 1996). Alternatively, increased IL-4 mRNA has been documented in biopsy specimens from early ileal lesions in some patients with Crohn's Disease (Desreumaux et al., 1997). Human recombinant IL-4 only, in the absence of immune cells did cause a small, but significant decrease in transepithelial ion resistance in naive T84 monolayers. This is consistent with the known ability of IL-4 to alter permeability in this model epithelium (Colgan et al., 1994) and confirms that the IL-4 was bioactive. However, using an experimental regime identical to that employed for IL-10, we found that IL-4 did not prevent SEB-induced epithelial abnormalities. At least 2 possibilities could explain the divergent effects of IL-10 and IL-4. First, IL-4 is an important differentiation factor for Th2 cells and as such may be less effective in modulating the functions of mature T cells in short-term (<24h) culture. Second, IL-4 may be a poorer inhibitor of Th1 cytokine production. Our data provide support for the latter hypothesis as IFN-γ and TNF-α production by SEB-activated immune cells was not affected by IL-4. Also, cytokine (IL-1β, IL-6, IFN-γ, TNF-α) production by daily treatment with 100 ng of IL-10 for 7 days significantly reduced the increase in T84 permeability elicited by subsequent exposure to IFN-γ or TNF-α. When these findings are compared to the data presented here, the intriguing scenario can be envisaged were IL-10 as an immuno-therapeutic agent may have dichotomous effects: early down-regulation of Th1 events, and a longer term benefit where the epithelium is rendered less susceptible to cytokines that can directly modulate permeability.

Anti-CD3 activation of PBMC (monocytes required) causes similar, but not identical changes in epithelial function to those evoked by SEB-activated T + B cells (monocytes not essential) (McKay and Singh, 1997; McKay et al., 1996b). In addition, SEB is more efficient in triggering the TcR (Viola and Lanzavecchia, 1996) and is a more potent enhancer of the TNF-α gene promoter in vitro (Kramer et al., 1995) than anti-CD3 antibodies. IL-10 was found to prevent the epithelial dysfunction caused by coculture with anti-CD3-activated PBMC, but not conditioned medium from these immune cells. This implies a common mechanism in the down-regulation of SEB and anti-CD3-induced epithelial pathophysiology. If this speculation is correct, then IL-10 may be of value in the treatment of a variety of T-cell mediated enteropathies (e.g. such as graft-versus-host disease) and transplant rejection (MacDonald and Spencer, 1992).

Bacterial infection and exposure to bacterial products/toxins can directly increase T84 permeability (Philpott et al., 1996). Having identified that IL-10 could prevent epithelial abnormalities caused by immune activation by bacterial (SEB) and immunological (anti-CD3) stimuli, we compared this with any putative ability to abrogate epithelial functional changes as a consequence of bacterial infection. In contrast to immune-mediated changes, IL-10 did not affect the increase in epithelial permeability caused EPEC infection. These data again testify that IL-10 has no immediate direct effects on epithelial barrier function. However, this does not negate the possible therapeutic benefit of IL-10 in the treatment of inflammatory conditions initiated by the entry of luminal antigen into the mucosa as a consequence of bacterial disruption of the epitheliums' barrier properties.
human PBMC in response to toxic shock syndrome toxin-1 (another S. aureus-derived SAg) was significantly reduced by IL-10 but not IL-4 (Krarauker, 1995). Similarly, antigen-stimulated synovial fluid mononuclear cells from patients with Lyme arthritis produced substantial amounts of TNF-α and IFN-γ that could be reduced by treatment with IL-10, but not IL-4 (Yin et al., 1997).

In conclusion, using an in vitro model we have shown: 1) that increased epithelial permeability and reduced stimulated ion transport evoked by superantigen (SEB) and anti-CD3-activated T cells can be prevented, at least partially, by IL-4, which prevents the immune-mediated changes in epithelial function; and 4) increased epithelial permeability due to E. coli (EPEC) infection was not abrogated by concomitant treatment with IL-10. Collectively these data provide additional support for the therapeutic use of IL-10 in the treatment of enteric conditions where there is perturbed epithelial function. As a cautionary caveat, we would add that the efficacy of IL-10 treatment will likely be influenced by the complement of immune cells at the site of insult and the underlying etiology (i.e. immune-mediated versus bacterial infection) of the disease.

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Send reprint requests to: Derek M. McKay, Ph.D., Intestinal Disease Research Programme, HSC-3NS, Department of Pathology, McMaster University, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5. E-mail: mckayd@mcmaster.ca.