CD4+ T cells mediate superantigen-induced abnormalities in murine jejunal ion transport

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McKay, Derek M., Michelle A. Benjamin, and Jun Lu. CD4+ T cells mediate superantigen-induced abnormalities in murine jejunal ion transport. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G29–G38, 1998.—The immunomodulatory properties of bacterial superantigens (SAgs) have been defined, yet comparatively little is known of how SAgs may affect enteric physiology. Staphylococcus aureus enterotoxin B (SEB) was used to examine the ability of SAgs to alter epithelial ion transport. BALB/c mice, severe combined immunodeficient (SCID, lack T cells) mice, or SCID mice reconstituted with lymphocytes or CD4+ T cells received SEB intraperitoneally, and jejunal segments were examined in Ussing chambers; controls received saline only. Baseline short-circuit current (Isc) indicates net ion transport and Isc responses evoked by electrical nerve stimulation, histamine, carbahol, or forskolin were recorded. Serum levels of interleukin-2 (IL-2) and interferon-γ (IFN-γ) were measured. SEB-treated BALB/c mice showed elevated serum IL-2 and IFN-γ levels, and jejunal segments displayed a time- and dose-dependent increase in baseline Isc compared with controls. Conversely, evoked ion secretion was selectively reduced in jejunum from SEB-treated mice. Elevated cytokine levels and changes in jejunal Isc were not observed in SEB-treated SCID mice. In contrast, SCID mice reconstituted with T cells were responsive to SEB challenge as shown by increased cytokine production and altered jejunal Isc responses that were similar to those observed in jejunum from SEB-treated BALB/c mice. We conclude that exposure to a model bacterial SAg causes distinct changes in epithelial physiology and that these events can be mediated by CD4+ T cells.

Staphylococcus aureus enterotoxin B; intestine

SUPERANTIGENS (SAgs) are low molecular weight peptides that are produced by a variety of bacteria (that can occur commonly or sporadically in the gut), viruses, and possibly some protozoan parasites (10). Studies with Staphylococcus aureus enterotoxins and other bacterial SAgs have shown that these peptides are potent stimulants of specific T cell subsets (17). Unlike conventional antigens, SAgs bypass the classical route of antigen processing and presentation and cross-link outside domains of major histocompatibility class II (MHC II) molecules with the variable portion of β-chain (Vβ) of the T cell receptor, binding beyond the antigen-specific site. This initial activation (proliferation, cytokine production, and increased cytotoxic activity) can be followed by a period of anergy or depletion of the appropriate Vβ+ T cells (10, 14, 17). Thus SAgs can activate up to 25% of the host’s T cells and therefore are potentially important and environmentally relevant inflammatory stimuli. In this context SAgs have been implicated in the pathophysiology of inflammatory and autoimmune disorders, such as rheumatoid arthritis, multisystem vasculitis (Kawasaki disease), and diabetes (8, 38). However, whereas the immunomodulatory properties of SAgs are being precisely defined, there is a dearth of information on the physiological consequences of exposure to SAgs, particularly in terms of gut function.

There is increasing evidence implicating bacteria in the pathophysiology of enteric secretory and inflammatory disorders (42). Recently it has been postulated that bacterial SAgs could be involved in inflammatory bowel disease (19). This hypothesis is not unprecedented. In the mid-1960s it was shown that dogs and rhesus monkeys treated with an S. aureus extract or partially purified S. aureus enterotoxin B (SEB) developed diarrhea and intestinal changes typified by loss of epithelial microvilli and mitochondrial destruction, a mucopurulent exudent, and varying degrees of lymphocytic infiltration (20, 33, 46). Many of these histological changes were apparent 2–8 h after treatment. The mechanism(s) of these changes in the gut remains undefined. In hindsight, it appears that these changes in gut morphology are temporally consistent with SEB-induced increased levels of circulating cytokines, such as tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) (34). It has also been shown that mice display a significant weight loss in response to SEB (27) and that treatment with the related enterotoxin, staphylococcal enterotoxin A, results in a rapid recruitment of lymphocytes to the epithelial compartment in the duodenum of rats (4). Moreover, we (32) and others (13) have recently found that functionally intact SEB can cross confluent monolayers of human epithelial cell lines (i.e., T84 and Caco-2) in vitro and murine intestine in vivo. In the former study we also reported that immune activation elicited by SEB resulted in increased epithelial permeability and diminished the secretory responsiveness of T84 monolayers (32).

Extrapolating from these studies, the present study is the first in a series of investigations to delineate the enteric consequences of exposure to SAgs, where epithelial electrolyte ion transport was used as an index of gut function. Our findings indicate that mice treated with SEB develop a self-limiting enteropathy characterized by a rapid onset of dramatic irregularities in epithelial ion transport and that these events can be mediated by CD4+ helper T cells. We speculate that given the correct environmental conditions and genetic background, exposure to SAgs could result in severe intestinal electrolyte abnormalities and so initiate or contribute to enteric secretory functional disorders. Furthermore, we contend that this model can be used to examine not only the effects of bacterial SAgs per se but
also the role of T cells and T cell subsets in the immunophysiological regulation of gut function.

MATERIALS AND METHODS

Animals and Experimental Treatment

BALB/c mice. Male mice (7–9 wk old) were purchased from Charles River animal suppliers (St. Constant, Quebec, Canada) and housed under conventional conditions for 1–2 wk before treatment with a single intraperitoneal injection of 1–100 µg SEB (Sigma Chemical, St. Louis, MO). With use of the limulus amoebocyte lysate assay (Sigma Chemical) our SEB stocks were found to be free of lipopolysaccharide contamination. After treatment (2–144 h) mice were killed by cervical dislocation. Time-matched PBS (vehicle for SEB administration)-treated mice served as controls.

SCID/beige mice. Mice were obtained from the breeding colony at McMaster University. To check for the presence of T cells 1) segments of gut and spleen were Formalin fixed and proceeded to wax, and 3-µm sections were immunostained with polyclonal anti-CD3 antibodies (Dako Diagnostics, Mississauga, ON, Canada) and 2) splenocytes (10^6/ml) were stimulated in vitro for 48 h with 1 µg/ml SEB or concanavalin A (Con A; Sigma Chemical), and proliferation was assessed by [3H]thymidine incorporation and interleukin-2 (IL-2) measured in the culture medium by ELISA (PharMingen, Mississauga, ON, Canada). Severe combined immunodeficient (SCID) mice (8–12 wk old) were injected with SEB (5 µg ip) and examined 4 or 48 h later. In additional studies, SCID mice were reconstituted with a mixed lymphocyte population and examined 4 or 48 h later. In other preparations the mixed spleen and lymph node cells were resuspended in RPMI medium, and 10^5 cells were added to 106 cells/0.2 ml PBS were administered intravenously to SCID mice (21). In other preparations the mixed spleen and lymph node cells were resuspended in RPMI medium (containing 10% FCS and antibiotics; GIBCO BRL, Burlington, Canada) and incubated for 2 h at 37°C on sterile petri dishes to allow the adherence of monocytes/macrophages. The nonadherent lymphocytes were recovered, and CD4^+ T cells were positively selected using magnetic cell sorting (MACs; Miltenyi Biotec, Auburn, CA); 10^7 lymphocytes were incubated with 10 µl of colloidal superparamagnetic microbeads conjugated to rat anti-mouse CD4 antibodies (L3T4, Miltenyi Biotec) for 20 min at 4°C, and the cell suspension was passed through an LS^+ separation column mounted in a Midi-Macs magnet. After a buffer wash (PBS, 2 mM EDTA, and 0.5% wt/vol BSA), the column was removed from the magnet and the CD4^+ T cells were eluted with cold buffer. The positively selected CD4^+ cells were resuspended in sterile PBS, and 15 x 10^6 cells/0.2 ml PBS were administered intravenously to SCID mice (samples of immune cells were analyzed by fluorescent-activated cell sorting and monoclonal antibodies to murine CD3, CD4, and Vµ%). Four weeks later the reconstituted SCID mice were challenged with SEB (5 µg) and jejunal segments examined in Ussing chambers 4 h later. We concentrated on 4 h post-SEB treatment (2–144 h) mice served as controls.

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Immune Activation

Splenocyte proliferation. Splenocytes were isolated and resuspended in RPMI medium, and 10^6 cells were added to each well of 96-well sterile culture plates at 100 ng/ml of SEB or Con A (as a positive control). Forty-eight hours later each well was pulsed with 1 µCi of [3H]thymidine (DuPont-NEN, Wilmington, DE) in 50 µl of fresh medium, and 18 h incubation at 37°C, cells were harvested onto glass fiber filters and radioactivity was determined in a scintillation counter (Becton-Dickinson, Mississauga, ON, Canada). The results are expressed as the stimulation index (SI), obtained by dividing the radioactivity count (counts per minute) from stimulated splenocytes that from nonstimulated splenocytes from the same mouse.

Cytokine production. At autopsy blood samples were collected and serum was stored at −70°C before measurement of IL-2 and IFN-γ levels. Cytokines were measured in duplicate in three serial dilutions by sandwich ELISA, using paired antibodies from PharMingen and following the instructions of the manufacturer. Also, lamina propria lymphocytes (LPLs) were isolated (using standard techniques) from control mice or mice treated with SEB (100 µg) 4 h previously and incubated at 37°C with or without SEB treatment (1 µg SEB/10^6 LPLs). Twenty-four hours later cell-free supernatants were collected and assayed for IL-2 and IFN-γ.

Intestinal Ion Transport

A 12-cm portion of jejunum was excised beginning at the ligament of Treitz and was divided into four segments. Each piece of tissue was mounted in an Ussing chamber (exposed surface area 0.6 cm^2) and bathed in 10 ml of warm (37°C), oxygenated Krebs buffer (in mM): 115.0 NaCl, 8.0 KCl, 1.25 CaCl2, 1.2 MgCl2, 2.0 KH2PO4, and 25.0 NaHCO3; pH 7.35 ± 0.02 (28). Some experiments were conducted in a modified buffer in which chloride ions were replaced with isethionate and acetate ions (43). Glucose (10 mM) was added to the buffer on the serosal side of the tissue, and this was osmotically balanced by 10 mM mannitol in the luminal buffer. The buffers were maintained at 37°C by a surrounding heated water jacket and circulated by a CO2/O2 gas lift. Tissues were short-circuited at zero volts using an automated voltage clamp (WPI Instruments, Narco Scientific, Mississauga, ON, Canada) and the short-circuit current (Isc, in µA/cm^2) was continuously monitored as an indication of net ion transport. The circuit was opened at intervals to obtain potential difference values (mV), and tissue conductance (G in mS/cm^2; indicates barrier to passive ion flow) was calculated according to Ohm’s law. After a 15-min equilibration period, baseline Isc and G were recorded.

Stimulated Ion Transport

Enteric nerves in each preparation were activated by an electrical transmural nerve stimulation (ETS, at 10 Hz, 10 mA, 0.5 ms for a total time of 5 s), and the peak in Isc was recorded. Subsequently, histamine (10^-4 M) or the cholinergic agonist carbamol (CCH, 10^-4 M; Sigma Chemical) was added to the serosal buffer of two jejunal segments from each mouse, and the maximum increase in Isc to occur within 5 min was recorded. All tissues were finally exposed to the adenosyl cyclase activator forskolin (10^-5 M; Sigma Chemical) and the change in Isc was recorded. Maximal doses of these known secretagogues were chosen because they elicit active Cl^- secretion via intracellular Ca^2+ or cAMP (2). Additional experiments were performed in which the jejunal ion transport response to forskolin was assessed without prior exposure to histamine or CCH.

G30 SUPERANTIGEN-INDUCED ENTEROPATHY

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MPO Activity

Mucosal scrapings of intestinal tissue were snap frozen in liquid N2 and stored at −70°C before assay. Myeloperoxidase (MPO) activity was determined in accordance with the protocol of Wallace et al. (47). Briefly, tissue was homogenized for 15 s in hexadecyltrimethylammonium bromide extraction buffer (ml/50 mg of tissue). One-milliliter samples of the homogenate were transferred to sterile Eppendorf tubes and centrifuged for 2 min at 1,200 rpm, and the supernatant was retained. Two hundred microliters of o-dianisidine solution were added to 7 µl of sample in 96-well plates (samples examined in triplicate), and three absorbance readings (460 nm) were immediately taken at 30-s intervals. One unit of MPO activity is defined as the quantity of enzyme required to convert 1 µmol of H2O2 to water per minute at room temperature.

Data Presentation and Analysis

Examination of all PBS-treated control mice showed no significant differences in any of the parameters examined in this study, and therefore, for illustrative purposes, they have been grouped together as a single control group (in some figures the control animals time-matched to the 4-h and 48-h post-SEB-treated mice are shown as individual groups). All data are expressed as means ± SE; n values are the number of mice in each experiment, in which 2–4 intestinal segments were used in the ion transport studies. Data were compared using one-way ANOVA followed by post hoc comparisons with Newman-Keuls test, where P < 0.05 was accepted as the level of statistically significant difference. Statistical analysis compared data from test mice with time-matched controls.

RESULTS

BALB/c Mice

After SEB treatment some mice displayed obvious piloerection and hunched posture, and when the mice were killed portions of the intestine were filled with fluid. Similar events have been reported after in vivo T cell activation via anti-CD3 antibodies (11).

SCID mice and SCID Reconstitution

Immunohistochemical staining of sections of jejunum or spleen for CD3 positivity confirmed the general absence of T cells in the SCID mice used in this study. Corroborating this observation, splenocytes from SCID mice failed to proliferate or synthesize IL-2 in response to in vitro SEB or Con A challenge (Table 1). Fluorescence-activated cell-sorting analysis revealed that the mixed splenocyte-mesenteric lymph node population used to repopulate SCID mice contained 34.5 ± 10.9% CD3+ T cells, of which 9 ± 1% were Vβ8+. After positive selection, mice were reconstituted with T cells that were >95% CD4+. Histological assessment of jejunal segments from reconstituted mice revealed numerous T cells (CD3+) and was reminiscent of normal BALB/c intestine: 18.4 ± 1.6, 0.4 ± 0.04 (P < 0.05 compared with other groups), and 15.9 ± 2.2 CD3+ T cells/villus crypt unit for BALB/c, SCID, and reconstituted SCID jejunum, respectively (n = 5). SCID mice challenged with SEB did not show fluid accumulation in the intestine, whereas all of the T cell-reconstituted mice (particularly those given the purified CD4+ cells) challenged with SEB developed diarrhea by 4 h postchallenge, with obvious fluid accumulation in the small intestine and an absence of food contents.

Immune Activation

Table 1. Comparison of BALB/c and SCID splenocyte proliferation and IL-2 production in vitro

<table>
<thead>
<tr>
<th>Mouse Treatment</th>
<th>Con A, 1 µg/ml</th>
<th>SEB, 1 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>55.3 ± 8.9</td>
<td>3.8 ± 0.7</td>
</tr>
<tr>
<td>BALB/c + 4 h SEB</td>
<td>86.7 ± 17.9</td>
<td>12.2 ± 2.8*</td>
</tr>
<tr>
<td>BALB/c + 48 h SEB</td>
<td>57.6 ± 5.7</td>
<td>11.0 ± 1.9*</td>
</tr>
<tr>
<td>SCID</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>SCID + 4 h SEB</td>
<td>1.1 ± 0.1</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>SCID + 48 h SEB</td>
<td>1.8 ± 0.4</td>
<td>2.0 ± 0.3</td>
</tr>
</tbody>
</table>

IL-2 production, ng/ml

<table>
<thead>
<tr>
<th>Mouse Treatment</th>
<th>Con A, 1 µg/ml</th>
<th>SEB, 1 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>6.4 ± 1.54</td>
<td>4.0 ± 2.6</td>
</tr>
<tr>
<td>SCID</td>
<td>0.04 ± 0.02*</td>
<td>0.01 ± 0.01*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4–6 mice. SCID, severe combined immunodeficient; IL-2, interleukin-2; Con A, concanavalin A; SEB, Staphylococcus aureus enterotoxin A. *P < 0.05 compared with control BALB/c mice.

SCID Mice. There was no increase in the SI in response to SEB or Con A in splenocytes from SCID mice with or without SEB treatment (Table 1). Splenocytes from reconstituted SCID mice did show a slightly enhanced immune responsiveness evoked by in vivo SEB treatment, such that in vitro proliferation to Con A was increased 3.5-fold (n = 4).

Cytokine production. BALB/c Mice. Four hours after SEB (5 µg) treatment, serum levels of IL-2 and IFN-γ were increased (Fig. 1); by 48 h posttreatment the levels of IL-2 and IFN-γ had returned to control values. Mice treated with high-dose SEB (100 µg) also displayed increased serum levels of IL-2 (60.2 ± 11.1 ng/ml) and IFN-γ (6.7 ± 1.7 ng/ml) 4 h after treatment that remained elevated at 48 h posttreatment (48.4 ± 13.8 and 3.8 ± 1.8 ng/ml for IL-2 and IFN-γ, respectively). LPLS isolated from mice treated 4 h earlier with SEB showed increased spontaneous and stimulated IL-2 and IFN-γ production in culture, compared with LPLS from control mice (Table 2).

SCID Mice. Neither IL-2 nor IFN-γ was increased in serum from SCID mice with or without SEB challenge, but both were apparent in serum obtained from SEB-treated CD4+ T cell-reconstituted SCID mice (Fig. 1). Serum from CD4+ T cell-reconstituted mice that were...
eliminated the increase in $I_{sc}$ observed in tissues from SEB-treated mice (Fig. 3), implicating active anion secretion as the cause of the elevated $I_{sc}$. Calculation of tissue ionic conductance ($G$) showed that this was significantly reduced 2 and 4 h after administration of 5 µg of SEB intraperitoneally (Table 3). Likewise, treatment with higher doses of SEB led to a drop in $G$ ($21.3 \pm 1.5$, $14.6 \pm 4.9$ (P < 0.05), and $13.7 \pm 3.4$ (P < 0.05) mS/cm$^2$ for jejunum from control mice and those treated with 100 µg SEB and examined 4 and 48 h later, respectively ($n = 4$).

**SCID MICE.** Baseline $I_{sc}$ in jejunum from SCID mice was very similar to that recorded in BALB/c mice (29.5 ± 3.7 mS/cm$^2$; $n = 10$; compare with Fig. 2). After SEB treatment, baseline $I_{sc}$ was slightly elevated to 40.7 ± 3.9 ($n = 10$) and 42.7 ± 9.6 mS/cm$^2$ ($n = 7$) at 4 and 48 h posttreatment, respectively; these increases were not statistically significant. Analysis of jejunum from SCID mice reconstituted with mixed lymphocytes or purified CD4$^+$ T cells 4 h after SEB (5 µg) challenge did not reveal a statistically significant increase in baseline $I_{sc}$; 32 ± 2.1 and 44.6 ± 4.0 mS/cm$^2$, respectively ($n = 5$ and 6 mice). Ion conductance in normal SCID jejunum was not affected by treatment of the mice with SEB. In contrast, jejunal tissues from SCID mice reconstituted with mixed lymphocytes or CD4$^+$ T cells and examined 4 h after in vivo SEB challenge showed dramatically reduced $G$ values (10.9 ± 1.1 and 11.5 ± 1.4 mS/cm$^2$; $n = 5$ and 6 mice) compared with tissues from control SCID mice (24.3 ± 1.7 mS/cm$^2$).

**Stimulated Ion Secretion**

ETS. **BALB/c MICE.** Jejunum from mice treated 2 or 4 h previously with low-dose SEB (5 µg) displayed a dramatically reduced (<50% of control values) secretory response to nerve stimulation (Fig. 4A). When mice were treated with higher doses of SEB the reduced responsiveness to electrical nerve stimulation was apparent 48 h after intraperitoneal administration of the SAg (Fig. 4B).

**SCID MICE.** The $I_{sc}$ evoked by ETS of enteric nerves in jejunal segments was not altered by SEB (5 µg) treatment of normal SCID mice but was significantly reduced in reconstituted SCID mice (Fig. 5).

**Ca$^{2+}$-evoked activeCl$^-$ secretion.** **BALB/c MICE.** Jejunal tissues from SEB-treated mice displayed a diminished $I_{sc}$ response when Ch was added to the serosal side of the tissue (Fig. 6A). This response was time dependent with the $I_{sc}$ being significantly attenuated 2 and 4 h after SEB treatment. The reduced response to Ch was also observed in jejunal segments examined 4 h after mice had been given a single intraperitoneal injection of 1, 50, or 100 µg SEB. In addition, higher doses of SEB prolonged the abnormal secretory nature of the epithelium, with diminished responses to Ch being evident 48 h after treatment (Fig. 6B). In contrast to the SEB-evoked alteration in the ability of the epithelium to respond to Ch, the secretory response to exogenous histamine at a maximal dose (10$^{-4}$ M) was not consistently altered at any time point (2–144 h posttreatment), irrespective of the administered dose (1–100

**Table 2. LPL in vitro cytokine production**

<table>
<thead>
<tr>
<th></th>
<th>IL-2, ng/ml</th>
<th>IFN-γ, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No stimulus</td>
<td>SEB</td>
</tr>
<tr>
<td>Control mice</td>
<td>0.75</td>
<td>0.82</td>
</tr>
<tr>
<td>SEB-treated mice</td>
<td>8.00</td>
<td>15.19</td>
</tr>
</tbody>
</table>

Data represent mean response of lamina propria lymphocytes (LPLs) from 3 mice/group (one representative experiment); ND, not detected. LPLs were isolated and cultured for 24 h ± SEB (1 µg/10$^6$ cells). Mice treated with SEB received 100 µg intraperitoneally and were killed 4 h later.
The diminished secretory response to forskolin was protracted and was still apparent 24 h after treatment with this low dose of SEB (data not shown). This diminished ability of the tissue to respond to forskolin was evident 2 h after a single intraperitoneal injection of 5 µg SEB. Unlike the abnormal secretory responses to ETS and CCh, the diminished secretory responses to forskolin in tissue that had not been previously exposed to histamine or CCh. Under these in vitro conditions, jejunal tissues from SEB-treated (5 µg) mice still displayed a ~60% reduction in the response to forskolin (49.9 ± 18.9 µA/cm² compared with 115.3 ± 18.2 µA/cm² in control tissues (n = 4 mice)).

SCID mice. As with the other prosecretory stimuli, forskolin-evoked changes in jejunal I_{sc} were very similar in tissues excised from SCID mice and SCID mice treated with 5 µg SEB 4 h previously. After reconstitution of SCID mice with the mixed lymphocyte population or purified CD4+ T cells, I_{sc} responses evoked by forskolin (after CCh or histamine exposure) were consistently reduced to 20–30% of control values.

SCID mice reconstituted with CD4+ T cells, but not challenged with SEB, displayed no signs of diarrhea and had a normal baseline I_{sc} and normal I_{sc} responses to secretagogues.

MPO Activity

There was no significant difference in MPO levels in jejunal homogenates from saline-treated control mice and SEB (5 µg)-treated mice autopsied 4–48 h later. MPO levels in jejunal homogenates from saline-treated control mice and mice examined 4 and 48 h after SEB treatment were not significantly different (Fig. 8).

Table 3. BALB/c jejunal baseline electrophysiological parameters

<table>
<thead>
<tr>
<th>Time After 5 µg SEB Challenge, h</th>
<th>0 (control)</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>144</th>
</tr>
</thead>
<tbody>
<tr>
<td>I_{sc}, µA/cm²</td>
<td>29.1 ± 2.5</td>
<td>36.9 ± 3.1</td>
<td>34.2 ± 2.2</td>
<td>43.8 ± 4.2</td>
<td>47.1 ± 6.1*</td>
<td>47.1 ± 4.5*</td>
<td>54.3 ± 4.2*</td>
<td>30.0 ± 3.6</td>
</tr>
<tr>
<td>PD, mV</td>
<td>-1.6 ± 0.2</td>
<td>-3.5 ± 0.2*</td>
<td>-2.9 ± 0.3*</td>
<td>-2.5 ± 0.4</td>
<td>-3.0 ± 0.5*</td>
<td>-3.2 ± 0.6*</td>
<td>-2.9 ± 0.8*</td>
<td>-1.4 ± 0.5</td>
</tr>
<tr>
<td>G, mS/cm²</td>
<td>24.0 ± 0.9</td>
<td>13.1 ± 1.1*</td>
<td>15.2 ± 1.3*</td>
<td>18.3 ± 2.7</td>
<td>18.8 ± 1.9</td>
<td>17.1 ± 0.9</td>
<td>21.2 ± 1.5</td>
<td>22.8 ± 2.2</td>
</tr>
<tr>
<td>n</td>
<td>30</td>
<td>7</td>
<td>18</td>
<td>7</td>
<td>10</td>
<td>7</td>
<td>14</td>
<td>5</td>
</tr>
</tbody>
</table>

Values are means ± SE. I_{sc}, short-circuit current; PD, potential difference; G, ionic conductance. * P < 0.05 compared with control.
The primary role of the immune response is the recognition and destruction and/or elimination of antigens and pathogens. However, it is also clear that inappropriate or exaggerated immune reactions can result in tissue damage and pathology (24). For instance, mice that lack the regulatory cytokine IL-10 develop a spontaneous enterocolitis, the severity of which is dramatically reduced when the animals are maintained under germ-free conditions (22). Indeed, microflora and/or bacterial products have been repeatedly implicated in enteric disease (42). Recently reported examples (7, 48) of this show that *Helicobacter*...
hepaticus and Cryptosporidium parvum infections enhance the gut inflammation that develops in SCID mice reconstituted with CD4$^+$, CD45RB high T cells and in mice lacking the $\alpha$-chain of the T cell receptor, respectively. Extrapolating from these observations, we hypothesized that exposure to a potent T cell activator, that is bacterial SAgs, could result in altered intestinal function. To test this hypothesis, we treated mice with SEB, as a model bacterial SAg, and subsequently examined epithelial ion transport as our key index of gut function. To summarize, we found that 1) exposure to SEB resulted in a time- and dose-dependent disruption of jejunal tonic and stimulated ion transport, 2) these events were not observed in mice devoid of T cells (i.e., SCID mice), and 3) abnormal ion transport events in response to SEB-immune activation could be reproduced in SCID mice by repopulating these animals with CD4$^+$ T cells. Thus we have shown that bacterial SAgs can perturb enteric ion transport and that T cells are important in this enteropathy.

In defining the immunomodulatory properties of bacterial SAgs, many studies have shown that T cells isolated from SEB-treated mice are hyperresponsive to in vitro SEB stimulation (6, 23). Therefore, before examining any intestinal physiological implications of exposure to SEB it was important to confirm that the SEB did affect the immune system in the treated mice. In accordance with the recognized immunostimulatory nature of SAgs, we observed that splenocytes from SEB-treated mice showed an enhanced proliferative response (i.e., increased $[^{3}H]$thymidine incorporation) to SEB in vitro compared with splenocytes from control mice. Confirmatory evidence of SEB causing an immune activation was provided by the increased levels of IL-2 and IFN-$\gamma$ in the serum of SEB mice compared with controls. In addition, LPLs from SEB-treated BALB/c mice produced greater amounts of IL-2 and IFN-$\gamma$ on secondary challenge with SEB in vitro; this is indicative of previous in vivo exposure (14). Having shown that SEB did have an immune effect, we proceeded to consider the enteric physiological ramifications of exposure to this model SAg.

Functional assessment of jejunal tissue revealed that baseline $I_{sc}$ was significantly increased by $\sim$40% 12–48 h after SEB treatment. Subsequent studies showed that this increase in tonic ion transport was most likely due to heightened anion secretion because it was not observed in tissues bathed in Cl$^-$-free buffer. Enhanced luminally directed anion secretion would create a driving force for water movement and so could initiate or contribute to a diarrheal response (39). Indeed, macroscopic examination often revealed an increased fluid content in the gut lumen of SEB-treated mice. Thus should SEB gain access to the mucosa it could elicit an immune cascade that could result in increased movement of water into the gut lumen. In this context, SEB has been found to cross monolayers of confluent human epithelial cells in vitro and murine intestine in vivo after oral administration (13, 32). In addition, the barrier function of rat jejunal epithelium is disrupted after exposure to a mild stress regimen (43). If the same is true of human intestine, then SAgs in the gut lumen could gain access to the mucosa after stressful life events and therefore evoke or augment enteric secretory or inflammatory disorders. This scenario may be particularly relevant in situations in which there are increased numbers of T cells and/or increased MHC II expression in the intestine (see Ref. 24).

Fig. 8. Changes in SCID jejunal $I_{sc}$ in response to exogenous histamine ($10^{-4}M$) added to serosal side of tissue (see Fig. 5 legend). *P < 0.05 compared with SCID.

Fig. 9. $\Delta I_{sc}$ evoked by forskolin (FSK; $10^{-5}M$) in jejunal tissues from BALB/c mice treated 4 or 48 h previously with various doses of SEB. A: $\Delta I_{sc}$ response to FSK in tissues that had previously been challenged with CCh ($10^{-3}M$). B: $I_{sc}$ response to FSK from tissues that had been previously challenged with histamine (Hist; $10^{-4}M$). *P < 0.05 compared with no SEB; n = 6–8.
In contrast to the increase in baseline \( I_{sc} \), jejunal segments from SEB-treated mice consistently displayed diminished \( I_{sc} \) responses to electrical nerve stimulation and exogenous CCh and forskolin. Diminished \( I_{sc} \) responses to these three prosecretory stimuli were evident in jejunal tissue from mice treated 2 or 4 h previously with 5 µg of SEB, and these abnormalities were prolonged in mice given higher doses of SEB (i.e., 50 or 100 µg). Although the exact mechanism and physiological importance of this diminished ion transport responsiveness is unknown, it is clear that exposure to SEB results in dramatic, but reversible, disruption of normal intestinal ion transport. Hystamine, similar to CCh, evokes active Cl\(^-\) secretion using intracellular Ca\(^{2+}\) as a second messenger (3). The changes in \( I_{sc} \) evoked by maximal doses of exogenous histamine were not significantly different in tissues excised from saline-treated controls and SEB-treated mice at any time point. This differential responsiveness of jejunal tissue from SEB-treated mice to secretagogues that utilize intracellular Ca\(^{2+}\) as a second messenger implies specific changes in the transporting enterocyte, rather than a general loss of epithelial secretory capacity. This postulate is supported by studies with other models of gut inflammation (18, 28, 39) or examination of resected inflamed human tissue (16, 40), where diminished secretory responses and differential responses to known secretagogues have been reported. In one such model where colitis was chemically induced in rats, a loss of Na\(^+\) and Cl\(^-\) absorption was correlated with epithelial destruction (5). Epithelial viability was not examined in the present study, but we have presented preliminary data showing altered enteric morphology in response to SEB (5a). It is still unclear as to how these changes in villus and crypt structure directly impact on the ion transport abnormalities. In addition we report that SEB treatment results in \( \sim 50\% \) drop in the magnitude of stimulated ion transport events, and this compares favorably with other enteropathies (e.g., mitomycin-induced colitis, parasitized rats) in which increases in \( I_{sc} \) evoked by nerve stimulation, substance P, prostaglandin E\(_2\), or serotonin were reduced by 30–75\% compared with responses in tissues from control animals.

Voluminous literature has accumulated illustrating the range of cells (immune, neural, and stromal) and messenger molecules that can regulate (enhance and downregulate) intestinal electrolyte transport (39). Because the T cell has been identified as an important cell in the mediation of SAg effects (23, 27, 29), we questioned the role of T cells in the current model of SAg-induced enteropathy. The SCID mouse (derived from a congenic partner of BALB/c) lacks mature functional T cells but does contain natural killer cells and cells of the monocytic lineage are reportedly normal (1). After confirming the general absence of T cells in the SCID mice used in this study (immunocytochemically and functionally), we observed that SEB treatment of these animals had minimal or no effect on jejunal ion transport. These data suggested a role for T cells in the SAg-induced epithelial abnormalities. To further test this postulate, SCID mice were reconstituted with a mixed population of spleen and/or mesenteric lymph node cells or purified CD4\(^+\) T cells. SEB challenge of these mice resulted in increased serum IL-2 and IFN-\(\gamma\) levels, and although CD3\(^+\) T cells were present in the gut of these mice, we do not suggest that these cells were the source of the IL-2, but rather that T cell reconstitution conferred the ability to respond to SEB (also supported by the enhanced responsiveness of LPLs from SEB-treated mice to in vitro SEB challenge; Table 2). Furthermore, on challenge with SEB (5 µg ip) reconstituted SCID mice displayed very similar, and on occasions more severe, alterations in ion transport compared with those observed in SEB-treated normal BALB/c mice. These data demonstrate a central role for CD4\(^+\) T cells in the mediation of SAg-induced epithelial ion transport abnormalities. Similarly, CD4\(^+\) cells have been implicated in other models of enteric dysfunction (9, 45). However, our data do not negate a putative role for CD8\(^+\) T cells in SAg-induced changes in gut function in normal mice. CD8\(^+\) cells do respond to SAg (10, 17, 23), and the role of these cells in SAg-induced enteropathy needs to be tested. A role for non-T cells in SAg-induced changes in the gut must also be considered (note small drop in \( \Delta I_{sc} \) to CCh in the jejunum of SEB-treated SCID mice). In this context, there is fragmentary evidence showing effects of SAg on MHC II\(^+\) monocytes and fibroblasts (35), mast cells (44), and dendritic cells (36). Thus, whereas we have shown an unequivocal role for CD4\(^+\) T cells in SEB-induced changes in epithelial ion transport, we do not dismiss the putative involvement of other cells, possibly working in concert with T cells, in the modulation of epithelial ion transport in response to SEB. Additionally, the selective administration of T cell cytokines (see below) to SCID mice may be of use in more fully elucidating T cell modulation of epithelial physiology.

The onset of the epithelial electrolyte transport abnormalities in this model of SAg enteropathy is fairly rapid. These findings complement those of Neumann et al. (37), who showed that SEB could evoke rapid (within 6 h) pathology in the lungs of mice, and earlier studies documenting enteric morphological and ultrastructural changes in response to SEB exposure (20, 33, 46). Although studies are in progress to more fully define the mechanism(s) underlying this irregular enteric ion transport, clues are available from the literature and our investigations with an in vitro model of SEB-induced epithelial (T84) transport and barrier dysfunction (32). For instance, levels of IL-2, IFN-\(\gamma\), and TNF-\(\alpha\) are elevated in murine serum 2–8 h after challenge with SEB and the related SAg, staphylococcal enterotoxin A (12, 34). These changes in cytokine levels are temporally consistent with the altered ion transport observed in the current study. Also, data from a variety of model systems show that IFN-\(\gamma\) and TNF-\(\alpha\) can directly or indirectly affect epithelial electrolyte transport (15, 26, 30, 41). Corroborating these findings we have recently described the ability of SEB-activated peripheral blood mononuclear cells to increase the permeability characteristics and reduce the secretary...
responses to CCh and forskolin in a model epithelium, namely monolayers of the human colonic T84 cell line (32). The epithelial ion transport dysfunction in this in vitro model is very similar to that observed in jejunal tissue excised from SEB-treated mice; reduced T84 secretory responsiveness is apparent 3–6 h after coculture with SEB-activated immune cells. Furthermore, neutralizing antibodies to IFN-γ and TNF-α were found to wholly or partially inhibit the epithelial abnormalities in the coculture model. Collectively these studies implicate IFN-γ and TNF-α as likely candidates in the mediation of the intestinal changes elicited in response to SEB. Further research efforts are required to confirm or refute this postulate.

A neutrophilic infiltrate is a common feature of acute inflammation. Neutrophils can contribute to tissue damage, and in vitro studies have documented the ability of these cells to evoke active Cl– secretion (25). In the present study, an elevation in MPO levels (as an indicator of granulocyte infiltration) was not observed. Thus we have shown that T cell activation, as evidenced by increased IL-2, can result in significant changes in epithelial ion transport in the absence of a neutrophilic infiltrate.

In summary, this study describes an enteropathy evoked by a model bacterial superantigen (SEB) that is characterized by alterations in electrolyte transport and occurs in the absence of any significant neutrophilic infiltrate. CD4+ T cells have been identified as an integral component in the mediation of altered epithelial ion transport in response to SEB, although the precise mechanisms (i.e., mediators, cell-cell signaling) underlying these events remain to be elucidated. We speculate that given suitable environmental conditions (e.g., T cell Vβ expression, altered barrier function of the epithelium, etc.) bacterial SAgs could contribute to the initiation or exaggeration of intestinal secretory abnormalities and may, in some patients with chronic or low-grade inflammation, lead to relapses in active disease.

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