Characterization of enteric functional changes evoked by in vivo anti-CD3 T cell activation

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Abstract

Specific in vivo T cell activation initiated by treatment with anti-CD3 antibodies leads to diarrhea and structural damage of the intestinal mucosa. In this study, the effect of T cell-induced mucosal damage on jejunal epithelial ion transport, muscle contractility, and neural ACh release was assessed in Ussing chambers, organ baths, and a specialized perfusion apparatus, respectively. Time-matched control mice received hamster serum containing irrelevant antibodies. Jejunal segments from anti-CD3-treated mice displayed a significantly elevated epithelial baseline short-circuit current (which indicates increased ion transport) and a concomitant reduction in responsiveness to prosecretory stimuli (nerve stimulation, carbachol, and forskolin). Longitudinal smooth muscle displayed altered spontaneous contractile activity, length-tension relationships, and carbachol-stimulated contraction in tissues excised from mice 20 and 40 h posttreatment. Anti-CD3 treatment did not affect stimulated ACh release from myenteric plexus neurons. We conclude that specific T cell activation via anti-CD3 antibody results in dramatic alterations in jejunal epithelial and smooth muscle function. Such T cell-induced changes in intestinal function may contribute to the symptomatology of T cell-mediated enteropathies, including graft-versus-host disease, celiac disease, and idiopathic inflammatory bowel disease.

EXAGGERATED OR INAPPROPRIATELY regulated immune events can result in significant alterations in intestinal structure and function. T cells have been implicated as a pivotal cell type in the mediation of intestinal pathophysiology (10, 21). For instance, intestinal mucosa resected from patients with inflammatory bowel disease (4) and celiac disease (23) have increased numbers of activated T cells. Experimental models of graft-versus-host disease, helminth infections in rodents, and analysis of human fetal intestinal explants have demonstrated that T cell activation alters gut structure (22, 35, 40). In contrast, there is significantly less information on the ability of T cells to regulate the physiological functions of the intestine.

Patients receiving immunosuppressive therapy with anti-CD3 antibody often develop a syndrome consisting of fever, headaches, and diarrhea (30). Similarly, administration of anti-CD3 antibodies to mice results in a "cytokine release syndrome" that is characterized by T cell activation, i.e., increased serum interleukin (IL)-2, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, and clinical diarrhea (13–15). We have recently reported that in vitro coculture of anti-CD3-activated peripheral blood mononuclear cells with a model human CI-secretory epithelial cell line (T84) evokes significant increases in epithelial permeability and a diminished capacity of the T84 monolayer to respond to prosecretory stimuli (27). In this in vitro system no change in baseline ion transport was detected. These observations indicate that specific T cell activation through the T cell receptor/CD3 complex alters epithelial physiology. However, the gut is a multifunctional organ and diarrhea is the net outcome of altered epithelial ion transport (secretion or absorption) creating driving forces for directed water movement, increased permeability, and/or dysregulated peristalsis. In addition, altered neuronal and stromal cell influences on the normal homeostatic responses can result in abnormal water movement and muscle contractility. Caution must be employed when extrapolating from in vitro data to the in vivo situation. There are a number of examples that illustrate how the in vivo situation can be very different from that predicted by in vitro experimental results. For example, the absence of CD4+ T cells did not prevent major histocompatibility complex class II-restricted T cell responses (32) and the effects of in vivo endotoxin on colonic fluid and electrolyte handling were different than those seen during in vitro experiments (5). Thus, although in vitro models are instructive, they must be supplemented by integrated in vivo observations.

Here we used an integrated, multisystem approach to define the enteric physiological ramifications of specific in vivo T cell activation. Changes in gut morphology, neuronal activity, epithelial electrolyte transport, and muscle contractility in response to specific in vivo T cell activation via treatment with anti-CD3 antibodies were examined. Our findings illustrate that the jejunum from mice treated with anti-CD3 display increased epithelial ion transport and altered spontaneous muscle activity and increased contractility of the longitudinal smooth muscle. This supports the postulate that in vivo T cell activation significantly alters gut function at a number of levels.

MATERIALS AND METHODS

Mice

BALB/c mice (female, 6–8 wk old; Charles River) received a single intraperitoneal injection of 50 µg of the monoclonal anti-CD3 antibody.
anti-CD3 antibody 145–2C11 prepared from hamster hybridoma cells (kindly provided by Dr. J. Bluestone, University of Chicago) in 0.5 ml of PBS. Control animals received an equal amount of nonspecific hamster IgG (H IgG) (Rockland, Gilbertsville, PA). Mice were housed in microfilter-isolated cages, allowed free access to autoclaved food and water, and were killed by cervical dislocation at various times after treatment. This study was approved by the McMaster University Animal Care Committee and conforms to the guidelines of the Canadian Council on Animal Care.

Histological Studies

Portions of jejunum were excised 1 cm distal to the ligament of Trietz and were immediately immersion fixed in 10% neutral-buffered Formalin. The tissue segments were then dehydrated though graded alcohols, cleared in xylene, and embedded in paraffin wax. Sections (3 µm) were cut perpendicular to the longitudinal axis of the villi, collected on coded slides, and stained with hematoxylin and eosin. Sections were examined for changes in mucosal architecture and evidence of neutrophil infiltrate.

Myeloperoxidase Determination

Tissues for assessment of myeloperoxidase (MPO) content were snap-frozen in liquid nitrogen and stored at −70°C. MPO was measured as previously described (3). MPO levels were determined as units of MPO per milligram of tissue wet weight, with one unit of MPO representing the amount of enzyme able to convert 1 mol of H2O2 to water in 1 min at room temperature.

Immune Activation

TNF-α levels. Serum samples were taken from mice killed at various time points after anti-CD3 treatment. TNF-α levels were measured in the serum using a commercial ELISA (R&D Systems, Minneapolis, MN). Each sample was tested in duplicate, and the concentrations were determined from a standard curve generated in each assay. The sensitivity of the TNF-α ELISA was 5 pg/ml.

Mucosal lymphocyte activation. Intraepithelial lymphocytes (IEL) were isolated from groups of five mice and pooled as previously described (9). Briefly, the small intestines were flushed with PBS, Peyer’s patches were excised, and IEL was released from the remaining tissue by incubation in Ca2+-Mg2+-free Hank’s medium (supplemented with 0.1 mM EDTA) for 30 min at 37°C with continuous stirring. IEL were purified by filtration through nylon wool columns followed by density centrifugation (600 g for 25 min) on a discontinuous Percoll gradient (40 and 70% (vol/vol)). The resultant IEL population was >95% viable and 80–90% CD3+ as determined by flow cytometry analyses performed as previously described (9). Contamination by lamina propria lymphocytes, as assessed by measuring surface immunoglobulin-positive cells, accounted for <10% of the preparation (9) and morphological assessment revealed <5% contamination by epithelial cells.

Mice received an injection of 1.5 mg ip of bromodeoxyuridine (BrdU) 4–6 h before autopsy. The isolated IEL and spleen cells were fixed in 70% ethanol at −20°C for 60 min and permeabilized, and the nuclear DNA was denatured into single-stranded molecules with 2 N HCl in 0.5% (vol/vol) Triton X-100 (Sigma, St Louis, MO) solution for 30 min at room temperature. Excess HCl was first neutralized with 0.1 M Na2B4O7 (pH 8.5) and then washed in 0.5% Tween 20–1.0% BSA-PBS (Tweek-BSA-PBS) solution (pH 7.4). Cells were immunoreacted with FITC-conjugated mouse anti-BrdU monoclonal antibody (Becton Dickinson) or FITC-conjugated isotype control antibody (both diluted 1:20 in Tween-BSA-PBS) for 30 min at room temperature. After further washing in Tweek-BSA-PBS, BrdU positivity was quantified by flow cytometry analysis (10,000 events were counted/sample) (9). BrdU positivity identifies cells that were activated into S phase of the cell cycle during the 4–6 h of exposure to BrdU.

Enteric Functional Changes

Epithelial Ion Transport. Assessment of murine jejunal epithelial ion transport was performed as previously described (25). Briefly, beginning 2 cm distal to the ligament of Trietz, two adjacent portions of full-thickness jejunum (~2.5 cm long) were excised and mounted in Ussing chambers (exposed surface area = 0.6 cm2). The jejunum was bathed in 10 ml of oxygenated Krebs buffer (in mM: 115.0 NaCl, 8.0 KCl, 1.25 CaCl2, 1.2 MgCl2, 2.0 KH2PO4, 25.0 NaHCO3, pH 7.35 ± 0.02). The buffers were maintained at 37°C by a surrounding heated water jacket and circulated by a CO2-O2 gas lift. Glucose (10 mM) was added to the buffer on the serosal side of the tissue and was osmotically balanced by the addition of 10 mM mannitol to the luminal buffer.

BASELINE SHORT-CIRCUIT CURRENT. Each tissue was 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/cm2 was continuously monitored as an indication of net ion transport. At 15-min intervals the voltage-clamp mode was removed, and the spontaneous potential difference (PD; in mV) of the tissues was measured. Tissue ionic conductance (Gm; in mS/cm2) was then calculated using Ohm’s law. Baseline Isc and Gm were measured after a 20-min equilibration period.

STIMULATED ISc Increases. Increases in ISc were evoked by transmural electrical stimulation of the enteric nerves (ETS at 10 Hz, 10 mA, 0.5 ms for a total time of 5 s) (25) and then by sequential addition of the cholinergergic agonist carbacol (10−4 M) and the adenylyl cyclase-activating agent forskolin (10−5 M) (both from Sigma). For the response to secretagogues, tissues were first exposed to carbacol, which caused a transient increase in ISc. Fifteen minutes later, when ISc had returned to baseline, forskolin was added to the serosal side of the same Ussing-chambered jejunal tissue and this evoked a sustained elevation of ISc. Epithelial responsiveness was defined as the maximum increase in ISc to occur within 5 min of nerve stimulation or exposure to the secretagogues. These agents and their maximal doses were chosen because they elicit predominantly an active Cl− secretion via intracellular Ca2+ and cAMP, respectively (1), and for direct comparison without previous in vitro investigations (27).

Smooth muscle contractility. Isometric tension and spontaneous contractile activity were recorded from muscles strips as previously described for mouse intestine (37). A 1-cm portion of jejunum was removed, oriented along its longitudinal axis, and placed in oxygenated (95% O2–5% CO2) Krebs solution. Tissue was aligned vertically with one end anchored to the base of the 20-ml bath and the other attached to a Grass FT03 force transducer. Responses were measured isometrically and recorded on a Grass 7D polygraph. The frequency and amplitude of spontaneous contractile activity were determined after a 30-min equilibration period, with 300 mg of tension. Intestinal tissues (>3/mouse) were stretched along their longitudinal axis by applying various tension settings (0–2,000 mg of weight), allowed to equilibrate for 10 min, and then contracted by exposure to carbacol (10−6 M). After stimulation, tissues were washed twice with Krebs, moved to the next tension setting, and reexposed to carbacol. At the end of each experiment, the tissues were blotted dried and weighed. Optimal tension was determined by the degree of
applied tension that produced a maximum contraction in response to carbachol. Tissue weight and length at the optimal tension was used to determine cross sectional area of tissue, as previously described (39). Dose-response experiments were also conducted for carbachol (10^{-4}–10^{-9} M), in which each tissue was immediately stretched to the optimal tension as determined by the length-tension experiments.

The force generated in the length-tension and dose-response curves is expressed in milligrams and normalized for cross sectional area (CSA) as determined by the following equation: $CSA \ (\text{mm}^2) = \frac{\text{tissue wet weight} \ (\text{mg})}{[\text{tissue length (mm)} \times \text{density (mg/mm}^3\text{)]}}$, where density of smooth muscle was presumed to be 1.05 mg/mm^3 (39). The concentration of the agonist required to induce a 50% maximum response (ED_{50}) was obtained by a probit analysis of Litchfield and Wilcoxon for each tissue, and a mean ED_{50} value was calculated for each mouse.

Neurotransmitter release from the myenteric plexus. Jejunal longitudinal muscle-myenteric plexus (LMMP) preparations were incubated for 60 min in oxygenated Krebs buffer containing [3H]choline (5 µCi). After washing, the tissues were mounted in a superfusion chamber, subjected to an electrical field stimulation; 30 V, 0.5 ms, 10 Hz for 60 s and subsequently to exposure to 50 mM KCl for 6 min. The evoked release of [3H]choline was determined as the difference between total [3H]choline released during stimulation and the calculated spontaneous release of [3H]choline from the tissue. This value was expressed as a fractional release unit (fru) and was calculated as a percentage of total [3H]choline present in the tissue at the time of stimulation. This assay for [3H]choline release was validated by our laboratory (7).

Data Presentation and Statistical Analysis

Data are presented as means ± SE, and n values represent the number of mice per group. Student’s t-test was used for statistical comparisons between two groups, and a one-way ANOVA followed by post hoc comparisons (Newman-Kuels test) was used to examined data from three or more experimental groups. A statistically significant difference was accepted at $P < 0.05$.

RESULTS

Jejunal Morphology

Mice treated with anti-CD3 were noticeably lethargic, developed a hunched posture, piloerection, and diarrhea, as shown by discoloration of the perianal area, within 4 h of treatment. This confirms previously reported effects of murine anti-CD3 treatment (9). Macroscopically, there was fluid accumulation in the small bowel of anti-CD3-treated mice, especially at 20 h postinjection. Histological examination of jejunal sections from anti-CD3-treated mice revealed dramatic changes in jejunal morphology. Twenty hours after treatment, villus height was reduced and the crypts appeared to have degenerated with varying degrees of edema and cell death, as evidenced by obvious pyknotic nuclei (Fig. 1D). These events were further exaggerated by 40 h posttreatment, when the villi were significantly stunted and normal crypts were virtually unrec-
ognizable (Fig. 1, E and F). The disorganized mucosa was littered with pyknotic nuclei, and released Paneth cell granules were often prominent. These changes in jejunal structure had resolved by 120 h post-anti-CD3 treatment. During this time course, there was no obvious jejunal neutrophil infiltration in response to anti-CD3. Corroborating this finding, tissue MPO levels were not elevated by anti-CD3 treatment: control vs anti-CD3. Corroborating this finding, tissue MPO levels were not elevated by anti-CD3 treatment: control vs anti-CD3. This change in baseline electrolyte transport was self-limiting and was not observed in tissues from mice 120 h after a single anti-CD3 injection. In contrast to the increase in basal ion transport tone, stimulated ion transport was significantly reduced in tissues from anti-CD3-treated mice. In response to electrical nerve stimulation, exogenous carbachol, (10^{-4} M) or forskolin (10^{-5} M), tissues from anti-CD3-treated mice showed diminished I_{sc} responses compared with jejunal segments obtained from control mice (Table 3). This diminished ion transport responsiveness was not evident 120 h after treatment. Transepithelial ionic conductance, indicating the barrier to passive ion movement, was not significantly altered at any time after anti-CD3 exposure (Table 3).

**Immune Activation**

TNF-α production. Anti-CD3-treated mice showed an increase in serum TNF-α that was maximal 30 min after injection and declined gradually thereafter. At 20 h after treatment, no significant difference was detectable between the anti-CD3-treated and the control group (Table 1).

**Enteric Functional Changes**

Epithelial function. When examined in Ussing chambers, jejunal segments from anti-CD3-treated mice displayed a statistically significant increase in baseline I_{sc}, reflecting an increase in active ion transport (Fig. 2). This change in baseline electrolyte transport was self-limiting and was not observed in tissues from mice 120 h after a single anti-CD3 injection. In contrast to the increase in basal ion transport tone, stimulated ion transport was significantly reduced in tissues from anti-CD3-treated mice. In response to electrical nerve stimulation, exogenous carbachol, (10^{-4} M) or forskolin (10^{-5} M), tissues from anti-CD3-treated mice showed diminished I_{sc} responses compared with jejunal segments obtained from control mice (Table 3). This diminished ion transport responsiveness was not evident 120 h after treatment. Transepithelial ionic conductance, indicating the barrier to passive ion movement, was not significantly altered at any time after anti-CD3 exposure (Table 3).

**Muscle function.** Because significant changes in jejunal epithelial ion transport were evident at 20 and 40 h posttreatment, we focused our subsequent studies on muscular activity on these two times. Muscle strips from control HIgG-treated mice exhibited a constant frequency and amplitude of contraction (Table 4). Twenty hours after anti-CD3 treatment, there was an increase in frequency of contraction and a reduction in amplitude (Table 4 and Fig. 3). By 40 h post-anti-CD3 treatment, the frequency of smooth muscle contraction had returned to normal, but the amplitude of contraction remained depressed (Table 4).

Significant differences were observed in the length-passive tension characteristics of the longitudinal smooth muscle, particularly at the lower tension settings, in tissues from mice 20 and 40 h after anti-CD3 treatment. Figure 4 shows the tension generated with carbachol at different applied tensions in tissues taken from mice 20 h after anti-CD3 treatment. The results show a significant increase in contraction from 0 to 750 mg of applied tension after stimulation with 10^{-6} M of carbachol. Jjejunal tissues from mice treated 40 h previously with anti-CD3 showed a carbachol-induced contractile response that was still significantly greater than that in control tissues but was slightly reduced compared with 20 h CD3-treated animals (data not shown). However, although the absolute response was different between test and control animals, muscle preparations from all groups generated the maximum

Table 1. Serum TNF-α levels after anti-CD3 treatment of BALB/c mice

<table>
<thead>
<tr>
<th>Time, h</th>
<th>HIgG</th>
<th>Anti-CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>12 ± 4</td>
<td>718 ± 90*</td>
</tr>
<tr>
<td>2</td>
<td>40 ± 22</td>
<td>520 ± 45*</td>
</tr>
<tr>
<td>4</td>
<td>10 ± 5</td>
<td>308 ± 18*</td>
</tr>
<tr>
<td>8</td>
<td>10 ± 5</td>
<td>242 ± 125*</td>
</tr>
<tr>
<td>20</td>
<td>4 ± 3</td>
<td>81 ± 8*</td>
</tr>
</tbody>
</table>

Values are mean serum levels of tumor necrosis factor-α (TNF-α) (pg/ml) ± SE for 3 experiments. HIgG, hamster IgG. *P < 0.05.

Table 2. Percentage of IEL and spleen cells incorporating BrdU after in vivo treatment with anti-CD3

<table>
<thead>
<tr>
<th></th>
<th>HIgG</th>
<th>Anti-CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>6.53 ± 2.7</td>
<td>7.9 ± 5.0</td>
</tr>
<tr>
<td>IEL</td>
<td>12.6 ± 5.4</td>
<td>36.1 ± 9.4*</td>
</tr>
</tbody>
</table>

Values are mean proportion of mononuclear cells ± SE that stain positive with FITC anti-bromodeoxyuridine (BrdU) antibody (see MATERIALS AND METHODS). Cells were isolated from mice 20 h after treatment with anti-CD3 antibody or HIgG control antibody. Data are derived from 3 separate experiments each with 5 mice/ experiment. IEL, intraepithelial lymphocytes. *P < 0.05.
increase in tension in response to 500 mg of applied
tension.

Muscle preparations from anti-CD3-treated mice had a significant increase in contraction generated in response to concentrations of carbachol \((10^{-6} \text{M})\) (Fig. 5). HIgG-treated mice maintained the same ED$_{50}$ values at 20 h \((1.43 \times 10^{-5} \pm 4.81 \times 10^{-6} \text{M})\) and 40 h \((1.43 \times 10^{-6} \pm 1.63 \times 10^{-6} \text{M})\). No significant difference in ED$_{50}$ values was observed between anti-CD3-treated mice at 20 h \((1.34 \times 10^{-5} \pm 1.62 \times 10^{-6} \text{M})\) and 40 h \((9.02 \times 10^{-5} \pm 3.21 \times 10^{-6} \text{M})\) compared with controls. As with the length-tension relationship, mice treated 40 h previously with anti-CD3 showed increased jejunal carbachol \((>10^{-5} \text{M})\)-evoked contraction compared with control tissues but were attenuated compared with their 20-h anti-CD3-treated counterparts (Fig. 5).

Neuronal function. Examination of stimulated \[^{3}H\]\textit{choline} release (as indicative of ACh release) from the LMMPPs revealed no significant differences between mice treated with anti-CD3 and their respective time-matched controls. LMMPPs from HIgG-treated mice had consistent release of radiolabeled ACh: 1.9 ± 0.2 and 1.9 ± 0.3 fru for 20 and 40 h time-matched controls, respectively. Anti-CD3-treated mice displayed no consistent deviation from control values: 20 h post-anti-CD3 = 1.8 ± 0.3 fru and 40 h anti-CD3 = 1.9 ± 0.3 fru. The response of anti-CD3-treated mice intestine to KCl stimulation was no different from control mice intestine (data not shown).

**DISCUSSION**

We report the results of an integrated study that examines the effects of specific in vivo T cell activation on several parameters of the physiological function of the small intestine. Our findings show that in vivo T cell activation via anti-CD3 crosslinking of the T cell receptor results in 1) increased jejunal epithelial baseline $I_{sc}$; 2) diminished ion transport responses to proinflammatory stimuli; and 3) altered spontaneous and carbachol-evoked (i.e., cholinergic) muscle contraction. In contrast, the stimulated release of ACh from myenteric neuronal plexus-longitudinal muscle preparations was not affected by in vivo anti-CD3 treatment.

Mice treated with a single intraperitoneal dose of the T cell-activating antibody anti-CD3 developed a clinical syndrome that includes severe self-limited diarrhea (14). Our present findings confirm previously documented structural changes of the small intestine associated with this syndrome: reduction in villus height and disruption of crypt structures (Fig. 1) (29). These changes are similar to those seen in intestinal diseases associated with T cell activation (4, 21, 23, 29). To document immune cell activation we showed that anti-CD3 treatment is associated with an increase in serum levels of TNF-$\alpha$ and with activation of local lymphocytes, as evidenced by the increased uptake of BrdU into IEL. Furthermore, we have previously shown that the anti-CD3 effect on gut architecture is dependent on T cells expressing the $\alpha/\beta$ T cell receptor, because mice lacking T cells (i.e., SCID mice, $\alpha/\beta$ T cell receptor knockout mice) fail to respond to anti-CD3 (29). Therefore this model allows us to determine the changes in epithelial and smooth muscle function that result from T cell activation and may contribute to the clinical syndrome of diarrhea associated with mucosal damage.

In response to anti-CD3 treatment, jejunal baseline $I_{sc}$ was increased about three- to fourfold 20–72 h after treatment. An increase in intestinal electrolyte transport is often indicative of electrogenic chloride transport into the gut lumen or, alternatively, enhanced cation (sodium) absorption (1). The in vivo observation of fluid accumulation in the intestinal lumen after anti-CD3 treatment in conjunction with increased basal $I_{sc}$ supports the contention that direct T cell activation results in a prosecretory or a malabsorptive diarrheal event. Therefore, in addition to neuroendocrine/immune factors that regulate water movement, specific T cell activation is also a contributing factor that may be relevant to the diarrhea experienced by patients with graft-versus-host disease, celiac disease, and other idiopathic disorders, such as inflammatory bowel disease.

The in vivo model also allows us to examine the changes in physiological function of a defined compart-

**Table 3. Jejunal ion transport after anti-CD3 or HIgG treatment of BALB/c mice**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HIgG Control</th>
<th>Post Anti-CD3 Treatment, h</th>
<th>F Value</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta I_{sc}$ to ETS, µA/cm²</td>
<td>63 ± 6</td>
<td>31 ± 9*, 21 ± 5*, 29 ± 7*</td>
<td>8.14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$\Delta I_{sc}$ to CCh, µA/cm²</td>
<td>106 ± 9</td>
<td>92 ± 9*, 15 ± 4*, 51 ± 16*</td>
<td>12.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$\Delta I_{sc}$ to FSK, µA/cm²</td>
<td>67 ± 12</td>
<td>42 ± 13, 33 ± 5*, 34 ± 5*</td>
<td>3.17</td>
<td>0.031</td>
</tr>
<tr>
<td>Conductance, mS/cm²</td>
<td>27.9 ± 2.1</td>
<td>24.2 ± 3.6, 26.3 ± 2.8, 31.9 ± 1.2</td>
<td>2.18</td>
<td>0.111 (NS)</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of mice. $\Delta I_{sc}$, change in short-circuit current; NS, not significant; ETS, electrical nerve stimulation; CCh, carbachol at $10^{-6}$ M; FSK, forskolin at $10^{-5}$ M. *P < 0.05 compared with intestine from HIgG mice.

**Table 4. Spontaneous contraction of longitudinal smooth muscle from anti-CD3 or HIgG-treated BALB/c mice**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Frequency of Contraction, Cycles/min</th>
<th>Amplitude of Contraction, cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIgG (20 h)</td>
<td>38.6 ± 0.3</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>HIgG (40 h)</td>
<td>38.5 ± 0.2</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Anti-CD3 (20 h)</td>
<td>43.4 ± 1.5*</td>
<td>0.4 ± 0.2*</td>
</tr>
<tr>
<td>Anti-CD3 (40 h)</td>
<td>38.4 ± 1.6</td>
<td>0.6 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 mice per condition. *P < 0.05 compared with time-matched HIgG control mice; measurements made at 300 mg of tension.
ment (e.g., epithelium) in the context of changes in other elements of the small intestine. For example, in our in vitro coculture studies, we did not observe an increase in baseline epithelial $I_{sc}$ after anti-CD3 activation of peripheral blood mononuclear cells (27). This indicates that other cells, such as fibroblasts (31), that are absent in the in vitro model likely modulate epithelial $I_{sc}$ after T cell activation. This illustrates the value of the integrative approach and the need to juxtapose in vitro and in vivo models when examining the influence of the immune system on the physiological function of the intestine.

In contrast to the changes in baseline $I_{sc}$, stimulated $I_{sc}$ responses were consistently reduced in jejunal segments from anti-CD3-treated mice. These changes correlate temporally with the alterations in gut morphology (i.e., villus atrophy, loss of crypt definition). This is similar to the results of our in vitro studies (27–29) as well as studies on gut segments from animals with enteric infections and tissues from patients with inflammatory bowel disease (19, 20, 25). Several scenarios could explain these changes in ion transport properties of the intestine. First, the reduced stimulated $I_{sc}$ responses may be a consequence of the increased basal $I_{sc}$. Basal ion transport is governed to a large extent by the enteric nervous system (8), thus it is feasible that tissues from anti-CD3-treated mice that display increased baseline $I_{sc}$ are somewhat tachyphylactic to neurotransmitters (i.e., electrical nerve stimulation and the cholinergic agonist carbachol). Second, decreased $I_{sc}$ responses could be due to epithelial cell loss and/or derangement of normal crypt architecture, because the crypt region is the predominant site of ion secretion. In support of this is the finding that chemically 2,4,6-trinitrobenzene sulfonic acid-induced colitis in rats causes major tissue damage and a concomitant loss of Na$^{+}$ and Cl$^{−}$ absorption (2). Third, the release of mediators such as arachidonic acid metabolites, reactive oxygen species, and cytokines from activated T cells or from other mucosal cells in response to T cell activation could directly modulate epithelial ion transport processes. In this context, in vitro studies with the T84 Cl$^{−}$ secretory epithelial cell line have shown that exposure to IFN-$\gamma$ or IL-4 can result in diminished secretory responses to forskolin, carbachol, and the neuropeptide vasoactive intestinal peptide (18, 27, 34, 41). Additionally, we have shown that T cell activation by a bacteria superantigen resulted in reduced T84 ion transport responses and that IFN-$\gamma$ and TNF-$\alpha$ were important mediators in this in vitro system (28). Indeed, both IFN-$\gamma$ and TNF-$\alpha$ have been implicated in the mediation of anti-CD3-induced diarrhea (26); however, our studies in knockout mice indicate that anti-CD3-induced disruption of normal gut structure can occur independent of TNF-$\alpha$ (29). Although the precise mechanism underlying the changes in ion transport in this anti-CD3-induced enteropathy remains to be elucidated, the present study illustrates the capacity of T cells to regulate directed water movement in the gut.
that jejunal segments from anti-CD3-treated mice were
had occurred in the smooth muscle tissue and this is
500 mg of tension for anti-CD3-treated and control
revealed that maximum muscle contraction occurred at
sponses. Analysis of the length-tension relationship
cant increase in carbachol-elicited contractile re-
We also found that anti-CD3 treatment caused a signifi-
cells in the regulation of muscle activity (6). This may
of gut tissue from parasitized rodents has implicated T
be mediated by cytokines such as IL-4 that can influ-
explore the ability of anti-CD3-activated T cells to
blood probe recovery studies are required to further
port and smooth muscle contractility that is not en-
treatment results in altered epithelial ion trans-
to smaller molecules (17, 36). Thus ex vivo flux studies
sitive increase in carbachol-elicited contractile re-
the gut to larger molecules (17, 36). Thus ex vivo flux studies with larger marker molecules and in vivo lumen-to-
thetic layers must also account for the barrier effect of the muscle layers and the outer
erosa, which do not confound studies using muscle-
mechanism responsible for this increase in spontaneous contractile activity may be related to effects on intermediate cells that regulate muscle activity, such as the putative pacemaker cells, the interstitial cells of Cajal (24).
To determine if anti-CD3 T cell activation induced the influx of granulocytes commonly seen during en-
teric infections, MPO activity was measured in tissue homogenates. We found no evidence for neutrophil infiltration in the intestine during the course of the enteropathy (29). Thus our data indicate that T cell activation results in rapid and distinct changes in jejunal structure and function in the absence of a classical marker of acute inflammation, the neutrophil.
In conclusion, we have illustrated that specific in vivo T cell activation results in altered epithelial ion transport and smooth muscle contractility that is not entirely predicted by in vitro studies using coculture systems or by studies of enteric infection-induced intestinal inflammation. These findings point to the value of in vivo studies where morphological and functional abnormalities resulting from changes in a number of systems can interact. Thus in vivo T cell activation leads to a small bowel enteropathy characterized by morphological and functional abnormalities reminiscent of those seen in graft-versus-host disease, celiac disease, and to some degree, inflammatory bowel disease. This model allows us to address the issue of defining the cellular and molecular mechanisms involved in the mediation of T cell regulation of enteric epithelial and smooth muscle function and gut homeostasis in general.

Perspectives
The traditional approach to understanding biological systems, where cells, mediators, and processes are considered as isolated distinct groups, is rapidly being replaced by a research approach where integration is emphasized. Although this seems intuitive when examining dynamic, multifunctional organs such as the gut, many studies focus on a single physiological process. In the present assessment of a murine T cell-driven enteropathy we examined the functional changes in a variety of target cells, i.e., epithelial cell, muscle cell, neuron, and granulocytes (as indicated by MPO activity). The changes in the behavior of these cells can independently, or cooperatively, result in abnormal gut function and, in this instance, diarrhea. In our in vivo model of T cell-mediated intestinal damage we have shown that epithelial electrolyte transport irregularities and altered muscle function without altered neurotransmitter release, contributed to the clinical syndrome of diarrhea. The precise role(s) of T cells, subsets of T cells, and chemical mediators released in response to specific T cell activation in immune-mediated gut dysfunction remain to be elucidated. Moreover, the broad, integrated research strategy employed here aptly illustrates the advantage of concomitant assessment of the activity of a variety of cell types when
attempts to dissect physiological pathways and understand pathophysiological mechanisms.

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