Nitric Oxide Participates in the Recovery of Normal Jejunal Epithelial Ion Transport Following Exposure to the Superantigen, *Staphylococcus aureus* Enterotoxin B

Derek M. McKay, Jun Lu, Sean Jedrzkiewicz, Winnie Ho and Keith A. Sharkey

*J Immunol* 1999; 163:4519-4526; [http://www.jimmunol.org/content/163/8/4519](http://www.jimmunol.org/content/163/8/4519)

**References** This article cites 41 articles, 24 of which you can access for free at: [http://www.jimmunol.org/content/163/8/4519.full#ref-list-1](http://www.jimmunol.org/content/163/8/4519.full#ref-list-1)

**Subscription** Information about subscribing to *The Journal of Immunology* is online at: [http://jimmunol.org/subscription](http://jimmunol.org/subscription)

**Permissions** Submit copyright permission requests at: [http://www.aai.org/About/Publications/JI/copyright.html](http://www.aai.org/About/Publications/JI/copyright.html)

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at: [http://jimmunol.org/alerts](http://jimmunol.org/alerts)
Nitric Oxide Participates in the Recovery of Normal Jejunal Epithelial Ion Transport Following Exposure to the Superantigen, Staphylococcus aureus Enterotoxin B

Derek M. McKay,* Jun Lu,* Sean Jedrzkiewicz,* Winnie Ho,† and Keith A. Sharkey†

Bacterial superantigens (SAgs) are potent T cell activators. Mice treated 4 h previously with the SAg, Staphylococcus aureus enterotoxin B (SEB), display reduced ion transport (assessed by short circuit current) responses to prosecretory stimuli, which normalize 24 h posttreatment. Here, mice were treated with SEB alone or in combination with an inhibitor of the inducible form of NO synthase (iNOS), L-NIL. Subsequently, jejunal iNOS expression was detected by immunohistochemistry, ion transport was evaluated in Ussing chambers, and serum levels of TNF-α and IFN-γ were measured by ELISA. SEB-treated mice had increased epithelial iNOS immunoreactivity, and numerous iNOS-positive CD3+ T cells occurred in their mucosa and submucosa. Concomitant treatment with L-NIL did not affect the reduced short circuit current responsiveness to electrical nerve stimulation or the prosecretory agents, carbachol and forskolin, that occurred 4 h post-SEB (5 μg) treatment. However, Isc responses in L-NIL plus SEB-treated mice were still significantly reduced 24 h posttreatment, indicating a role for NO in the restoration of normal ion transport following exposure to SAgs. The prolongation of epithelial ion transport abnormalities correlated with elevated serum levels of TNF-α and IFN-γ in mice treated 24 h previously with L-NIL plus SEB compared with those in controls and SEB-only-treated mice. Additionally, mice treated with L-NIL plus SEB and TNF-α or IFN-γ-neutralizing Abs displayed normal jejunal ion transport characteristics 24 h posttreatment. We conclude that NO mobilization is important in the homeostatic recovery response following immune stimulation by SAgs and that the beneficial effect of NO in this model system is probably via regulation of TNF-α and IFN-γ production. The Journal of Immunology, 1999, 163: 4519–4526.

Copyright © 1999 by The American Association of Immunologists 0022-1767/99/$02.00

Received for publication April 26, 1999. Accepted for publication July 29, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by research funding from the Medical Research Council of Canada and the Crohn’s and Colitis Foundation of Canada (to D.M.M. and K.A.S.). D.M.M. is a Medical Research Council of Canada Scholar, and K.A.S. is an Alberta Heritage Foundation for Medical Research Senior Scholar.

2 Address correspondence and reprints requests to Dr. Derek M. McKay, Intestinal Disease Research Program, McMaster University, Hamilton, Ontario, Canada; or Neuroscience and Gastrointestinal Research Groups, Department of Physiology and Biophysics, University of Calgary, Calgary, Alberta, Canada.

3 Abbreviations used in this paper: SAg, superantigen; Vβ, variable portion of the β-chain; MHC class II Ags; SEB, Staphylococcus aureus enterotoxin B; iNOS, inducible NO synthase; L-NIL, L-α-(1-iminoethyl)-lysine hydrochloride; SNAP, S-nitroso-N-acetylpenicillamine; bNOS, brain NOS; Isc, short-circuit current; G, tissue conductance; ETS, electrical transmural stimulation.

Materials and Methods

Animals and experimental treatment

Male BALB/c mice (7–9 wk old; Charles River, St. Constant, Canada) were maintained under standard housing conditions with free access to...
food and water. After a 1- to 2-wk acclimatization, experimental mice received 5 μg of SEB (Sigma, St. Louis, MO) by a single i.p. injection in 200 μl of sterile PBS (some mice received 100 μg of SEB i.p.). This treatment regime evokes time-dependent alterations in jejunal structure and function (6, 7). To examine a role for NO in these events, mice were treated with the selective inhibitor of iNOS, 1-NAME (1-iminoethyl)-lysine hydrochloride (t-NAME; RBI, Natick, MA; 100 or 200 μg i.p. in 200 μl of PBS) 30 min before SEB exposure (the specific role of an iNOS inhibitor was examined based on data from the immunohistochemical portion of this study (see below); other NOS activity inhibitors, such as Nω-nitro-l-arginine methyl ester (t-NAME) or Nω-nitro-l-arginine (t-NA), affect both constitutive and inducible forms of the enzyme and so were not employed here). Time-matched control animals received saline only. Four, 24, or 48 h after SEB treatment, mice were sacrificed by cervical dislocation, and segments of jejunum were removed. In additional experiments, mice were treated with monoclonal neutralizing Abs against TNF-α or IFN-γ (both at 100 μg/mouse in 100 μl of PBS i.p.; PharMingen, Mississauga, Canada) (13) concurrently with t-NIL (200 μg in 100 μl of PBS i.p.) and were treated with SEB (5 μg i.p.) 30 min later. Jejunal segments were examined 24 h post-SEB administration, and the responses were compared with those from mice treated with SEB only or with t-NIL plus SEB.

Before SEB exposure, mice were treated with the NO donor, S-nitroso-N-acetylpenicillamine (SNAP; RBI); each mouse received a total of either 20 or 100 μg of SNAP, administered in four equal i.p. doses at 60-min intervals over a 4-h period beginning 15 min before SEB treatment. The doses of t-NIL and SNAP used in this study are based on previous reports (15–17).

These studies were approved by the animal care committee at McMaster University and comply with the guidelines of the Canadian Council on Animal Care.

Immunohistochemistry

A midabdominal incision was made, the small intestine was exposed, and portions of jejunum (4 cm distal from the ligament of Treitz) were excised, rinsed in cold (4°C) PBS, and then fixed in Zamboni’s fixative (24 h at 4°C). Whole-mount preparations or cryostat sections (12 μm) were stained in PBS and incubated with primary Abs against either the constitutive brain NOS (bNOS), 1/1000 (Santa Cruz Biotechnology, Santa Cruz, CA); iNOS, 1/500 (Transduction Laboratories, Lexington, KY)) for 48 h at 4°C. After washing, tissues were incubated in donkey anti-rabbit IgG conjugated to CY3 (1/100; Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 h at room temperature. Tissues were washed, mounted in bicinecato-buffered glycerol (pH 8.6), and examined using a Zeiss Axiohot fluorescence microscope (Carl Zeiss, New York, NY) (18). In subsequent studies, double-immunostaining procedures were conducted to determine whether the iNOS-positive cells were T cells (i.e., CD3 positive; using anti-human polyclonal anti-CD3 primary Ab at 1/200; Dako, Mississauga, Canada) (6), macrophages (CD11b positive; using anti-mouse monoclonal M1/70 at 1/50; Serotec, Kidlington, U.K.) (19) or neutrophils (using anti-mouse neutrophil clone 7/4 at 1/500; Serotech) (20). For double-labelling protocols, appropriate FITC or CY3 conjugated secondary Abs were used (1/50; Jackson ImmunoResearch Laboratories).

Functional studies

Jejunal epithelial ion transport was assessed in Ussing chambers as previously described (7). Briefly, beginning at the ligament of Treitz, a 12-cm portion of jejunum was excised and divided into four segments. Each segment was mounted between the Lucite halves of a Ussing chamber (exposed surface area, 0.6 cm²) and bathed in 10 ml of oxygenated Krebs buffer (115.0 mM NaCl, 8.0 mM KCl, 1.25 mM CaCl₂, 1.2 mM MgCl₂, 2.0 mM KH₂PO₄, and 25.0 mM NaHCO₃, pH 7.35) at 37°C. After washing, the tissues were transferred to the chambers and equilibrated for 30 min. Experimental series were performed in duplicate in three serial dilutions, and the detection limit of each assay was 4 pg/ml.

Analysis

Data were presented as the mean ± SEM, where n is the number of mice examined (data from two to four tissues are averaged for each mouse in the Ussing chamber studies). Data were analyzed by one-way ANOVA followed by post-hoc statistics with the Newman-Keuls test, and a level of statistically significant difference was accepted at p < 0.05.

Results

SEB treatment results in increased iNOS expression

Immunohistochemical labeling of frozen sections of jejunum from control mice revealed negligible bNOS and iNOS expression in the epithelium or lamina propria. In both frozen sections and whole mount preparations there was a moderately dense bNOS innervation of the submucosal plexus (Fig. 1D) and iNOS-immunoreactive nerve fibers in the submucosa. Inducible NOS was not observed in the submucosa in control mice (Fig. 1A). In contrast, mice treated with either 5 or 100 μg of SEB displayed obvious iNOS expression, which occurred as a patchy distribution in the epithelium (data not shown) and as discrete cellular staining in the submucosa (Fig. 1). Qualitatively the pattern of iNOS immunoreactivity evoked by either 5 or 100 μg of SEB was similar at 4 and 48 h posttreatment (n = 4–6 mice/group). Double immunostaining of jejunal tissues from SEB-treated mice for iNOS and CD3 T cells revealed that the majority of iNOS-positive cells were T cells (Fig. 2). Moreover colocalization studies examining iNOS immunoreactivity and macrophage surface markers failed to reveal significant up-regulation of iNOS expression in these cells (only a few macrophages expressed iNOS, as judged by double labeling; Fig. 3). Also, SEB treatment did not cause increased iNOS expression in neutrophils (Fig. 3).

Inhibition of iNOS activity prolongs epithelial functional abnormalities

Given the rapidity of the induction of enhanced iNOS expression and our previous results that SEB treatment resulted in diminished secretory responsiveness to prosecretory stimuli by 4 h posttreatment (7), we hypothesized that NO might mediate these changes in jejunal epithelial function. However, treatment with neither 100 nor 200 μg of l-NIL consistently or significantly affected the reduced Isc responses observed 4 h after SEB (5 μg) treatment (Table I).

The alterations in jejunal ion transport elicited by low dose SEB resolve by 24 h posttreatment; however, the coadministration of l-NIL with SEB resulted in prolongation of the ion transport abnormalities. Twenty-four hours after SEB (5 μg) treatment jejunal
baseline Isc was slightly elevated, and this was significantly enhanced when L-NIL (200 μg) was combined with SEB: control, 29.5 ± 2.6 μA/cm² (n = 6); SEB, 37.8 ± 2.7 (n = 8); L-NIL, 39.2 ± 6.4 (n = 5); L-NIL plus SEB, 50.0 ± 2.6 (n = 7; * p = 0.04 compared with control; mean ± SEM). Similarly, baseline jejunal ion conductance was not different from tissues in control and SEB-treated mice were compared, but was significantly reduced in tissue excised 24 h after L-NIL plus SEB treatment (control, 20.1 ± 2.7 ms/cm²; L-NIL plus SEB treatment, 10.1 ± 3.8 mS/cm²; p = 0.04). Increases in jejunal Isc evoked by ETS (Fig. 4a), carbachol (Fig. 4b), and forskolin (Fig. 4c) were all significantly reduced by at least 50% in tissues excised from L-NIL plus SEB-treated mice compared with controls, SEB-only-treated, and L-NIL-only-treated mice. Jejunum from mice treated with L-NIL displayed ion transport characteristics that were not significantly different from those of PBS-treated control mice (Fig. 4).

FIGURE 1. Fluorescence photomicrographs of iNOS immunoreactivity (A–C) and bNOS immunoreactivity (D–F) in jejunal whole-mount preparations from control mice (A and D) and mice treated 4 h (B and E) or 48 h (C and F) previously with SEB (100 μg i.p.). Preparations were single labeled using CY3-conjugated secondary Abs. The number of iNOS-immunoreactive cells was significantly increased after SEB treatment. A similar increase in iNOS-immunoreactive cells was observed 4 h post-SEB treatment and also occurred in response to treatment with 5 μg of SEB (data not shown). The bNOS immunoreactivity was mainly localized in the submucosal plexus and was similar in all animal groups. Scale bar = 50 μm.

FIGURE 2. Fluorescence photomicrographs showing colocalization of iNOS and CD3 immunoreactivity (i.e., T cells) in jejunal whole-mount preparations from two SEB-treated mice (100 μg i.p., 4 h posttreatment). Preparations were double labeled using the combinations of Abs described in Materials and Methods, such that iNOS was labeled with an FITC-conjugated secondary Ab, and the immune cells were labeled with a CY3-conjugated secondary Ab. Mouse 1: A, iNOS immunoreactivity; B, CD3 immunoreactivity; mouse 2: C, iNOS immunoreactivity; B, CD3 immunoreactivity (arrowheads indicate iNOS⁺/CD3⁺ cells; arrows indicate iNOS⁺/CD3⁻ or iNOS⁻/CD3⁺ cells). Scale bar = 50 μm.
i-NIL effects are negated by concomitant treatment with anti-TNF-α or anti-IFN-γ Abs

Mice treated with i-NIL plus anti-TNF-α or anti-IFN-γ Abs and then SEB (n = 4) displayed Isc responses to all three prosecretory stimuli that were not significantly different from control values (Fig. 5).

SNAP does not alter the jejunal response to systemic SEB treatment

Treatment with 20 or 100 μg i.p. of the NO-donating compound SNAP (pilot studies showed that these doses of SNAP did not cause significant or consistent changes in the treated animals behavior; mice were notably lethargic when given 200 μg of SNAP i.p.), did not affect the diminished Isc responses to ETS, carbachol, or forskolin that occur 4 h post-SEB administration (Table II). SNAP activity (3 μM) was confirmed by direct addition to the serosal side of jejunum from control mice mounted in Ussing chambers, which resulted in an increase in Isc of 16.3 ± 8.9 μA/cm² (n = 8 tissues).

Altered jejunal architecture is not affected by iNOS inhibition

Mice treated with i-NIL only displayed a jejunal villus-crypt architecture that was not significantly different from that in control mice (Fig. 6). In contrast, there was a significant increase in crypt depth 24 h post-SEB treatment, while villus height was not different from time-matched control values. Jejunal morphology in mice treated with i-NIL plus SEB was not significantly different from SEB-only-treated mice. As a consequence of the crypt elongation in SEB-treated and i-NIL- plus SEB-treated mice, the villus-crypt ratio was reduced to 2.1 ± 0.1 (n = 5; p < 0.05 compared with control and i-NIL only) and 2.3 ± 0.2 (n = 6; p < 0.05 compared with control and i-NIL only), respectively, compared with jejum from control (2.9 ± 0.1; n = 5) or i-NIL-only-treated (3.1 ± 0.3; n = 3) mice.

i-NIL treatment results in sustained TNF-α and IFN-γ levels in response to SEB

Significantly elevated levels of TNF-α and IFN-γ (8.16 ± 4.1 and 4.3 ± 1.1 ng/ml, respectively (n = 3)) occurred only in serum samples obtained from mice treated 24 h previously with i-NIL plus SEB. Serum from mice treated with SEB (5 μg) only or i-NIL only and time-matched controls had negligible or undetectable levels of serum TNF-α (0–0.5 ng/ml) and IFN-γ (0–2.0 ng/ml; n = 2–3). The low dose of SEB used here does not result in animal death (6, 7), and coupling the SAg-stimulus with i-NIL also did not result in any mouse mortality over the 24-h experimental period.

Discussion

Extensive research efforts in a variety of models of inflammation or gut dysfunction have variously ascribed beneficial (21, 22) or detrimental (23, 24) roles to NO, with one study in captive rhesus...
macaques failing to identify any effect of inhibition of NO synthesis in modulating the spontaneous colitis that develops in these animals (25). The lack of consistency in these studies is most likely a reflection of the multifunctional character of NO, which is dependent on the synthesizing enzyme (i.e., cNOS vs iNOS), the cellular source (e.g., epithelial cells vs infiltrating neutrophils) (26), and the form of the NO (i.e., NO$_2^-$ vs NO) (27). Initial cytokine responses to SAg challenge typically have a Th-1 type profile (7, 28), and we previously identified roles for IFN-$
abla$ and TNF-$
abla$ in the alteration of epithelial function in vitro following coculture with SAg-activated T cells (8). Because Th-1 type cytokines can regulate NO production (and vice versa) (29–31), we postulated that NO was involved in the mediation of the effects of SEB in the gut. This hypothesis is not unprecedented (13), and our findings indicate an important role for NO in the recovery of normal gut electrolyte transport (the driving force for directed water movement) following immune stimulation by SEB.

Immunohistochemical studies revealed increased jejunal iNOS, but not bNOS (i.e., constitutive NOS), expression in response to SEB. Inducible NOS immunoreactivity was evident in jejunal epithelial cells (patchy distribution) in SEB-treated mice, and this is consistent with reports describing increased epithelial iNOS expression in response to bacterial infection or exposure to bacterial products (32, 33). Colocalization studies revealed that numerous, but not all, CD3$^+$ T cells had increased iNOS expression, and this would be predicted, because only a subpopulation of T cells will possess the appropriate TCR V$\beta$-chain capable of binding SEB (3). This increase in iNOS expression is in accordance with the findings of other studies showing that in vivo T cell activation via SEB or an anti-CD3 Ab results in increased serum nitrite/nitrate levels, the stable end products of NO metabolism (13, 34). While
enhanced epithelial iNOS expression has been described, few studies have documented iNOS activity in T cells in general (35), and our findings are the first demonstration of a mucosal T cell iNOS response to SAg treatment. Also, double-labeling studies revealed that the iNOS+ cells were not neutrophils, and this is consistent with data we previously reported showing that SEB treatment does not result in any significant jejunal neutrophilia (6). Furthermore, and perhaps initially surprising, iNOS expression in macrophages (i.e., CD11b+ cells) was not increased following SEB treatment. However, bacterial SAgs are recognized as mainly potent T cell activators, and to our knowledge, induction of iNOS expression in response to SEB ligation of MHC II on APC has not been demonstrated in any model system.

The marked up-regulation of iNOS expression implied a role for NO in mediating the response to SEB in the murine jejunum. In assessing the role of NO in changes in jejunal structure (altered villus-crypt morphology) and function (i.e., diminished responses to prosecretory stimuli), we postulated that iNOS-derived NO might mediate the rapid changes in gut function that are apparent 4 h post-SEB treatment. Alternatively, NO could be involved in the recovery of normal function after exposure to SEB (i.e., 24 h posttreatment). l-NIL treatment did not affect the diminished Isc responses to nerve stimulation or the Ca2+ or cAMP-mediated prosecretory agents, carbachol, forskolin, 4 h post-SEB treatment. In contrast, jejunal tissue excised 24 h posttreatment with l-NIL plus SEB displayed irregular ion transport characteristics that were not observed in tissue from time-matched control mice or animals treated with SEB or l-NIL alone. Thus, 24 h post-SEB treatment, basal ion transport (i.e., baseline Isc) was significantly increased, and responsiveness to ETS and carbachol was dramatically diminished. Additionally, the reduced response to forskolin observed in tissue from mice treated 24 h previously with SEB was potentiated by concomitant l-NIL administration. This disruption of normal tonic (i.e., baseline) and stimulated ion transport will affect water flow across the intestine, with repercussions for hydration of the gut surface and the mucosal defense that fluid secretion provides in the gut. Prolongation of altered jejunal ion transport with l-NIL plus SEB treatment indicates that mobilization of NO is an important component of gut homeostasis. Furthermore, it is likely that the NO in this model was derived from iNOS, because only increased iNOS immunoreactivity was detected, and l-NIL is reputedly a specific inhibitor of iNOS (17). In accordance with the data demonstrating a beneficial role for NO in ameliorating the enteric physiological abnormalities evoked by SEB, it has been shown that NO can protect against SEB-induced toxic shock (13) and anti-CD3 (i.e., T cell mediated)-induced murine mortality (34) and reduce the effects of S. aureus infection (29, 36).

As reviewed by Farthing and co-workers, NO has been ascribed both prosecretory and proabsorptive abilities (37). For instance, Ussing chamber studies have shown that gut tissue treated with NO-donating compounds can respond with an increase in active ion transport (38), whereas addition of l-NIL to tissues excised from mice with a chemically induced colitis partially prevents the reduced Isc responses to forskolin (39). Intestinal ion transport is closely regulated by the enteric nervous system (40), allowing for the possibility that the neurotransmitter function of NO might account for its role in the recovery of normal prosecretory responsiveness. Alternatively, it has been shown that SEB treatment results in rapid synthesis of TNF-α and IFN-γ (13, 28), and that inhibition of NO synthesis results in prolonged elevation of these proinflammatory cytokines. Indeed, in the present study elevated TNF-α and IFN-γ levels were only apparent in serum from mice treated 24 h previously with l-NIL plus SEB. Both these cytokines can affect epithelial ion transport (9). Indeed, in vitro studies have shown that model epithelia exposed to recombinant TNF-α and IFN-γ display a significant reduction in their responsiveness to carbachol, forskolin, and other prosecretory stimuli (9). To examine further the putative mechanism of NO modulation of the gut response to systemic SAg treatment, the l-NIL plus SEB experiments were repeated in mice that also received neutralizing TNF-α or IFN-γ Abs. Treatment with either Ab antagonized the effect of l-NIL, resulting in jejunal Isc responses of normal magnitude 24 h posttreatment. These findings support the postulate that the beneficial effect of NO in this system is via inhibition of TNF-α and IFN-γ production. A similar mechanism of NO action has been proposed in SAg-induced toxic shock (13).

Identification of the beneficial effect of NO in the recovery of normal jejunal ion transport characteristics after exposure to SEB

Table II. The NO-donating compound, SNAP, does not affect reduced jejunal ion transport events observed 4 h after SEB treatment*

<table>
<thead>
<tr>
<th></th>
<th>ΔIsc to ETS</th>
<th>ΔIsc to CCh</th>
<th>ΔIsc to FSK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.4 ± 3.6</td>
<td>68.6 ± 2.2</td>
<td>197.7 ± 24</td>
</tr>
<tr>
<td>SNAP (20 µg)</td>
<td>34.2 ± 11.5</td>
<td>67.2 ± 16.7</td>
<td>94.6 ± 42.3</td>
</tr>
<tr>
<td>SEB</td>
<td>16.5 ± 3.2*</td>
<td>38.6 ± 7.9*</td>
<td>69.7 ± 11.9*</td>
</tr>
<tr>
<td>SNAP (20 µg) + SEB</td>
<td>14.3 ± 6.8*</td>
<td>38.1 ± 11.7*</td>
<td>54.6 ± 3.8*</td>
</tr>
<tr>
<td>SEB</td>
<td>8.9 ± 1.7*</td>
<td>26.0 ± 7.3*</td>
<td>44.5 ± 12.6*</td>
</tr>
<tr>
<td>p (ANOVA)</td>
<td>&lt;0.001</td>
<td>=0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Jejunal tissues were examined in Ussing chambers 4 h after SEB (5 µg, i.p.) treatment and SNAP was given (i.p.) as four equal doses at 60-min intervals beginning 15 min before the SEB treatment (mean ± SEM; n = 3–5 mice/group; ETS, electrical transmural stimulation, ΔIsc, change in short circuit current in μA/cm²; CCh, carbachol at 10-6 M; FSK, forskolin at 10-5 M.

*, p < 0.05 compared with control.

FIGURE 6. Bar charts showing jejunal villus height (A) and crypt depth (B) in control mice and mice treated with l-NIL (200 µg i.p.), SEB (5 µg i.p.), and l-NIL plus SEB (200 µg i.p. of l-NIL 30 min before 5 µg i.p. of SEB; mean ± SEM; n = 3–5; by ANOVA, p = 0.021; *, p < 0.05).
presented the possibility that delivery of NO might inhibit the reduced secretory responsiveness that occurs 4 h post-SEB treatment. Experiments with the NO donor, SNAP (15–17), failed to significantly alter the early SEB effects in the murine jejenum. At least three scenarios can account for this observation. First, SNAP may not have liberated NO. This is unlikely, because SNAP added directly to Ussing-chambered tissue caused a transient increase in Isc, similar to that described for other NO donors (38). A second possible explanation for the discrepancy between the in vitro and in vivo effects of SNAP is that the rate or route of NO delivery was insufficient, or that the NO released did not reach the target cell to ameliorate the SEB-induced changes in ion transport. Third, the role of NO in the recovery of normal ion transport after SEB-evoked immune activation may be inextricably linked to other mediators that are produced in the cascade of events that occur in response to SEB-evoked immune activation; these mediators would not be present in the mucosa early in the treatment regimen, rendering excess NO ineffective. In this context, Rangachari and co-workers, in their assessment of the effects of isoprostanes on ion transport in the canine colon, have elegantly shown that the impact of these rapidly synthesized mediators is dependent on other mediators and the sequence of mediators to which the tissue has been exposed (41). Therefore, the role of NO and indeed that of other mediators also may differ under physiological vs pathophysiological conditions, and data defining the roles of these molecules must be interpreted in the context of the model system employed.

In addition to functional jejunal changes, SEB-treated mice display a slight reduction in villus height and increased crypt depth 4–12 h posttreatment (6). The present study extends these observations, showing that villus height was not significantly different from that in controls, although the crypts were still elongated (~20% increase) 24 h post-SEB treatment. Moreover, L-NIL plus SEB treatment neither exaggerated nor prevented the increase in jejunal crypt depth. The differential effects of L-NIL in modulating SEB-induced changes in jejunal structure and function indicate the mobilization of NO-dependent and NO-independent events following SAg exposure. The factors responsible for the change in gut architecture have not been examined, although growth factors and PGs are likely candidates, because both can directly affect the epithelium and influence villus-crypt morphology (42, 43).

Finally, in exploring the mechanism by which SEB, a model bacterial SAg, can affect murine enteric physiology and morphology, the present study demonstrates 1) that there is increased iNOS expression in T cells in the mucosa and submucosa; 2) that treatment with the blocker of iNOS activity, L-NIL prolongs the functional, but not the structural, changes evoked by SEB; and 3) that the effects of iNOS inhibition can be overcome by neutralization of TNF-α or IFN-γ. Thus, in contrast to the prevailing dogma that supports a deleterious role for iNOS-derived NO, our findings indicate that NO is involved in the recovery of normal jejunal ion transport after SAg-induced immune activation. Furthermore, the interaction of NO and TNF-α and IFN-γ has been demonstrated, with the beneficial effects of NO in this model most likely due to interference with TNF-α and IFN-γ production and/or activity.

References