Do eicosanoids cause colonic dysfunction in experimental E coli 0157:H7 (EHEC) infection?

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Abstract

Background—The pathophysiology of enterohaemorrhagic Escherichia coli (EHEC) infection remains unclear. Eicosanoids have been implicated as pathophysiological mediators in other colitides. Aims—To determine if prostaglandin E, (PGE,) and leukotriene B, (LTB,) contribute to mucosal inflammation and dysfunction in EHEC colitis.

Methods—Ten day old rabbits were infected with EHEC. For five days after infection, mucosal synthesis of PGE, and LTB, was measured in distal colonic tissue from control and infected animals and 51Cr-EDTA permeability was assessed in vivo. Myeloperoxidase activity was measured and histological inflammation and damage were assessed at five days in control and infected animals and after treatment of infected animals with the LTB, synthesis inhibitor MK-886. In separate experiments, ion transport was measured in Ussing chambers, before and after in vitro addition of the cyclooxygenase inhibitor indomethacin.

Results—LTB, synthesis was increased from day 2 after infection onwards and PGE, synthesis was increased on day 3. Mucosal permeability did not increase until day 5 after infection. MK-886 inhibited colonic LTB, production but did not reduce diarrhoea, inflammation, or mucosal damage. Electrolyte transport was not significantly altered on day 3 after infection. However, both Cl secretion and reduced Na absorption found on day 5 were partially reversed by indomethacin.

Conclusions—Tissue synthesis of PGE, and LTB, did not correlate temporally with EHEC induced inflammation or changes in mucosal permeability and ion transport. Cyclooxygenase inhibition partially reversed ion transport abnormalities but lipooxygenase inhibition did not affect mucosal inflammation or histological damage. We conclude that the contribution of eicosanoids to mucosal injury and dysfunction is more complex than previously suggested.

Keywords: enterohaemorrhagic Escherichia coli; electrolyte transport; prostaglandins; leukotrienes; chloride secretion

Since their recognition as human pathogens in 1983,1 enterohaemorrhagic Escherichia coli (EHEC) have been found to be responsible for outbreaks and sporadic cases of diarrhoea, haemorrhagic colitis, and haemolytic-uraemic syndrome in North America, the UK, Japan, and Australia.5 6 We have previously demonstrated that EHEC infection of infant rabbits reproducibly results in diarrhoea, disruption of colonic electrolyte transport, and histological damage of the colonic mucosa.9 Sodium absorption is gradually abolished and chloride secretion develops abruptly five days after infection.7 However, the factors which mediate colonic dysfunction in EHEC infection remain largely unknown.

Supposed microbial virulence factors, such as shiga-like toxins, fimbriae, and the ability of the bacteria to produce attaching and effacing lesions, have not been convincingly implicated in the pathogenesis either by us9 or others.6 With regard to the contribution of host-defence factors to EHEC pathogenesis, we previously showed that neutrophils are important cellular mediators of structural and transport changes.7 In this study we used this well characterised model to test current paradigms for the pathophysiology of infectious colitis.10 Specifically, we wished to investigate the potential contribution of eicosanoid mediators to colonic inflammation, injury, and mucosal dysfunction in EHEC infection.

Eicosanoids have been proposed as important pathophysiological mediators in experimental colitis.11 14 Levels of both cyclooxygenase and lipooxygenase products are increased in inflamed colonic mucosa in human inflammatory bowel disease15 16 and in animal models of colitis.17 18 Moreover, eicosanoids are recognised neutrophil products and have been linked to disruption of electrolyte transport19 20 and to tissue injury.12 14 21

Prostaglandin E (PGE,) has previously been demonstrated to be a potent and pivotal secretagogue in mammalian intestine.22 23 PGE, itself stimulates chloride secretion24 and, in addition, mediates the secretagogue effects of other components of the inflammatory milieu.25 26 Its role as an intestinal secretagogue has been convincingly demonstrated in monkeys infected with Salmonella27 and, more recently, in porcine cryptosporidiosis.11 It has also been suggested that PGE, mediates the increased mucosal permeability induced by exposure of ileal loops to Clostridium difficile toxin A.13

Abbreviations used in this paper: EHEC, enterohaemorrhagic Escherichia coli; PGE, prostaglandin E; LTB, leukotriene B, Cr-EDTA, chromium-ethylidiaminetetraacetic acid; MPO, myeloperoxidase; PD, potential difference.
Although some lipoxygenase products, such as 5-hydroxyeicosatetraenoic acid (5-HETE) and 5-hydroperoxyeicosatetraenoic acid (5-HPETE) also affect colonic electrolyte transport, their predominant role in colitis is in the pathogenesis of mucosal inflammation and injury.12 14 23 26 Leukotriene B$_4$ (LTB$_4$), for example, is a potent chemotactic factor affecting leukocytes.22 Thus prostaglandins and leukotrienes have been incorporated into current concepts of the pathophysiology of infectious diarrhoea and assigned central roles.10

The aim of this study was to test these concepts by defining the role of eicosanoids in the pathophysiology of EHEC colitis in infant rabbits. We studied the temporal relationships between tissue PGE$_2$ synthesis and ion transport changes and between mucosal LTB$_4$ synthesis and tissue inflammation and damage. The contributions of PGE$_2$ and LTB$_4$ were further examined using inhibitors of eicosanoid synthesis. While cyclooxygenase products partially accounted for changes in ion transport, the results suggest a complex interplay among elements of the inflammatory milieu rather than any clear cut “cause and effect” role for either PGE$_2$ or LTB$_4$ in EHEC induced mucosal dysfunction.

Methods

ANIMAL MODEL
As previously described, litters of suckling 10 day old New Zealand white rabbits, free of diarrhoea, were inoculated intragastrically with 5x10$^8$ CFU of EHEC strain EDL933 (serotype O157:H7, producing shiga-like toxins 1 and 2 and plasmid mediated fimbrial adhesin), suspended in 1 ml of 10% sodium bicarbonate buffer.6 7 Control rabbits were inoculated with 1 ml of bicarbonate buffer alone. Pups were kept with their mothers, who were allowed free access to chow and water. Animals were maintained on a 12 hour light/dark cycle. Pups were weighed daily and checked for perianal fur soiling to assess the development of diarrhoea. Infection was confirmed by plating rectal swabs onto sorbitol MacConkey agar and examining sorbitol negative colonies for the presence of O157 antigen by the slide agglutination method (E coli latex kit, Oxoid Ltd, Hampshire, UK).19 20 The use and care of animals were approved by the animal ethics committee of the Children’s Medical Research Institute.

STUDY PROTOCOLS
(1) To assess the time course correlations between mucosal eicosanoid levels and measures of mucosal inflammation, damage, and function, mucosal synthetic capacities for PGE$_2$ and LTB$_4$ were measured in control (uninfected) rabbits and in infected animals from day 1 to day 5 after inoculation.

(2) To assess the contribution of LTB$_4$ to colonic inflammation and mucosal damage, infected animals were treated with intramuscular MK-886, an inhibitor of LTB$_4$ synthesis. MK-886 blocks LTB$_4$ production by inhibiting activation of 5-lipoxygenase through inhibition of enzyme translocation.28 30 MK-886, dissolved in 1% carboxymethylcellulose, was administered (10 mg/kg/day) two hours before infection and then daily for five days after infection. Body weight gain, severity of diarrhoea, distal colonic LTB$_4$ synthesis, distal colonic myeloperoxidase (an index of mucosal inflammation), and histology score were measured five days after infection. Results were compared with data from control and infected animals not treated with MK-886. Vehicle treatment alone did not affect any measured parameter in infected animals (data from this group not shown).

(3) To assess the contribution of PGE$_2$ to alterations in ion transport, distal colonic electrolyte transport was studied in vitro before and after addition of the cyclooxygenase inhibitor indomethacin in control animals and infected animals on days 3 and 5 after infection. The dose used (final concentration 10$^{-6}$ M dissolved in ethanol) completely abolished PGE$_2$ release in this preparation. Pilot studies showed that the vehicle (ethanol, final concentration 1:100 vol:vol) did not significantly alter unidirectional or net ion fluxes or electrical parameters when added to day 3 (n=5) or day 5 (n=4) infected distal colon (data not shown). Indomethacin was added in vitro as it causes intestinal inflammation in vivo.

CLINICAL PARAMETERS, HISTOLOGY SCORE AND MYELOPEROXIDASE ACTIVITY
Clinical parameters were assessed in control (uninfected) rabbits and in infected rabbits 1–5 days after inoculation. Body weight gain (g) (over five days) was calculated from daily weight data. Severity of diarrhoea was scored according to the following criteria: 0, no soiling; 1, slight soiling around the anus; 2, moderate soiling of the perineum; and 3, severe, covering the perineum and extending down both legs.

For histological assessment of mucosal inflammation and damage, segments of distal colon were removed, fixed in 4% buffered formalin, and blocked in paraffin. Sections were then stained with haematoxylin and eosin and examined by light microscopy by a single observer blinded to the experimental treatment. Scoring of colonic inflammation and mucosal damage is depicted in table 1 and is a modification of the schema reported by Rath and colleagues.31

Myeloperoxidase (MPO), an index of tissue neutrophil infiltration and inflammation, was measured as previously reported. Briefly, scraped mucosa from the distal colon was homogenised in hexadeyltrimethylammonium bromide buffer and sonicated for five seconds. Samples were snap frozen in liquid nitrogen and assayed within three days for MPO activity, as described by Krawisz and colleagues.32 Results are expressed as units per gram of mucosa.
MUCOSAL EICOSANOID SYNTHETIC CAPACITY
Mucosal synthetic capacity for PGE, and LTB, was assayed as previously described in control
and infected animals from day 1 to day 5 after infection. Samples of distal colon (500–200
mg) were placed in Eppendorf tubes containing 1.5 ml of warmed 10 mM sodium phosphate buffer, pH 7.4. After mincing with scissors for 15 seconds, samples were incubated at 37°C for 20 minutes in a shaking water bath and centrifuged at 1600 g. The supernatant was stored at −20°C and eicosanoid content was assayed within two weeks using commercial enzyme immunoassays (Cayman Chemical Co., Ann Arbor, Michigan, USA). After thawing, samples for LTB, determination were centrifuged and boiled to degrade inter-
fering substances prior to immunoassay. Measurement of colonic eicosanoid synthesis
by this method has been shown previously to correlate well with measurements via in vivo
rectal dialysis in an animal model of colitis.

MUCOSAL PERMEABILITY MEASUREMENT
Permeability was assessed as a measure of mucosal function and as an additional index of
mucosal damage. In vivo permeability to 51Cr labelled ethylenediaminotetraacetic acid (51Cr-
EDTA; DuPont) was studied in control rabbits and in day 2–5 EHEC infected rabbits using
ligated colonic loops. In rabbits anaesthetised
with intraperitoneal ketamine and xylazine, a
carotid artery cannula was placed for blood
sampling. The abdomen was opened and the
renal pedicles were ligated. A cannula was
placed in the proximal transverse colon and the
colon distal to this was gently flushed with
Krebs buffer warmed to 37°C. After draining
the colon of buffer, a distal colonic loop was formed by placing silk ligatures at the distal
transverse colon and just above the rectum.
Care was taken to avoid excessive handling of
the colon and interruption of the vascular supply. The colon was kept moistened with saline
during construction of the loop. After tying the
distal ligature, 100 µCi of 51Cr-EDTA in 400 µl
of Krebs buffer were injected into the loop via
polyethylene tubing introduced through the
tightened proximal ligature. The tubing was
then withdrawn as the ligature was tied. The
abdomen was closed and the rabbit placed on a
heating pad for the remainder of the study.

Some of the 51Cr-EDTA/Krebs solution (50
µl) was reserved for gamma counting (to estimate total activity of the instilled 400 µl)
and arterial blood was drawn into heparinised syringes at 0 and 30 minutes. Blood samples
were immediately centrifuged and 100 µl
plasma samples were set aside. After sample
collection, the rabbit was killed and the length
of the excised loop was measured. 51Cr
activities of the instilled buffer and the 30
minute plasma sample were subsequently
determined by gamma spectrometry. Lumen to
blood permeability was calculated as a percent-
age of the amount of 51Cr-EDTA instilled into
the loop, which appeared per ml of plasma in
30 minutes, normalised to the length of the
loop.

COLONIC ELECTROLYTE TRANSPORT STUDIES
Transport studies were performed on excised
distal colon from control animals and EHEC
infected rabbits three and five days after infec-
tion. Electrolyte transport was studied in vitro
under voltage clamped steady state conditions
as previously described. Animals were killed
cervical dislocation. The colon was immedi-
ately removed, opened along the mesenteric
border, and gently washed of feces. Two or
three adjacent patches of unstripped colon
from each rabbit were mounted in leucite Uss-
ing chambers with exposed surface areas of
0.27 cm2. Each side of the tissue was bathed in
Krebs/glucose buffer (containing (in mmol/l)
Na+ 140, K+ 10, Mg2+ 1.1, Ca2+ 1.25, Cl− 127.7,
HCO3− 25, H2PO4− 2.0, and glucose 10; pH
7.4), maintained at 37°C by water jacketing of
the glass reservoirs and oxygenated and
circulated by means of a carbogen gas lift. Each
side of the leucite chamber was connected via
agar/KCl bridges to voltage electrodes and to
an automatic voltage clamp apparatus (DVC
1000, World Precision Instruments, New
Haven, Conncticutt, USA). 22Na and 36Cl (5
μCi) were added to either the mucosal or sero-
colal chamber to allow unidirectional flux calcu-
lations. After equilibration for 30 minutes,
basal period short circuit current (Isc, in
µEq/cm2/h), potential difference (PD, in mV),
conductance (G, in mSiemens/cm2), and
steady state unidirectional fluxes (J, in
µEq/cm2/h) of Na and Cl were measured during three consecutive 10 minute periods.
Indomethacin was then added to both sides of
the tissue and, after 35 minutes of further equilibration, electrical and flux measures were
repeated. Tissue pairs were discarded if conductance differed by more than 25%.

STATISTICAL ANALYSIS
Data are expressed as mean (SEM). Compara-
tions between data obtained at di-
quent multiple comparisons using Fisher’s

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Score</th>
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<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>No damage</td>
<td></td>
</tr>
<tr>
<td>Slight depletion of goblet cell</td>
<td></td>
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<tr>
<td>mucus; architecture and</td>
<td></td>
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<tr>
<td>epithelium intact; mild</td>
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<tr>
<td>apoptosis of surface colonocytes</td>
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<tr>
<td>(scattered cells).</td>
<td></td>
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<tr>
<td>Majority of goblet cells depleted</td>
<td></td>
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<tr>
<td>of mucus; moderate apoptosis</td>
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<tr>
<td>(clusters of apoptotic bodies).</td>
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<tr>
<td>All goblet cells depleted of</td>
<td></td>
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<tr>
<td>mucus; apoptotic cells</td>
<td></td>
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<tr>
<td>throughout epithelium;</td>
<td></td>
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<tr>
<td>microscopic ulceration of</td>
<td></td>
</tr>
<tr>
<td>surface epithelium</td>
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</tr>
<tr>
<td>PMN per high power field</td>
<td>None</td>
</tr>
<tr>
<td>Mucosal thickness (µm)</td>
<td>Control thickness &lt;25% thicker than control 25–50% thicker than control &gt;50% thicker than control</td>
</tr>
</tbody>
</table>
Eicosanoids and colonic dysfunction in E coli infection

MUCOSAL EICOSANOID SYNTHESIS

Mucosal synthetic capacity for PGE₂ was significantly higher in day 3 infected colon than in control colon or day 1 or day 2 infected tissue (fig 1A). Mean PGE₂ levels remained high on days 4 and 5 after infection but were not significantly different from control values (using ANOVA with post hoc testing). Mucosal LTB₄ synthetic capacity was significantly elevated on days 2, 4, and 5 after infection relative to values in control and day 1 infected colon (fig 1B).

MUCOSAL PERMEABILITY

In vivo distal colonic "¹¹Cr-EDTA permeability was studied as an additional index of mucosal damage and as a measure of mucosal function. There were no changes in paracellular permeability until day 5 after infection (fig 1C). In day 5 infected rabbits, colonic "¹¹Cr-EDTA permeability was significantly increased, being 60–100% higher than levels in control and other infected animals. The increase in mucosal permeability did not correlate with the time course of changes in PGE₂ or LTB₄ levels (fig 1) but corresponded temporally with the development of histological damage, onset of Cl secretion, and infiltration of the mucosa by neutrophils (fig 1, lower panels, as reported previously in this model).

COLONIC INFLAMMATION: EFFECT OF INHIBITION OF LTB₄ SYNTHESIS

As expected, MK-886 treatment inhibited tissue synthesis of LTB₄ (table 2). Infected rabbits treated with MK-886 gained significantly less weight over five days than control animals (table 2). However, MK-886 did not prevent the development of diarrhea in infected animals. MK-886 treatment did not diminish the significant increases in mucosal MPO activity and histological damage and inflammation induced by EHEC infection (table 2).

COLONIC ELECTROLYTE TRANSPORT: EFFECT OF INHIBITION OF PGE₂ SYNTHESIS

Electrolyte transport was measured in distal colonic tissue from control rabbits and from infected animals three and five days after infection (table 3). Under basal conditions, control distal colon actively absorbed Na and, at a lower rate, Cl. PD and Isc values were high, consistent with predominantly electrogenic Na absorption. In day 3 infected tissue, flux values and electrical parameters were not significantly different from controls with the exception of the serosal to mucosal (s→m) Cl flux, which was slightly but significantly lower. By day 5 after inoculation with EHEC, Na absorption was markedly reduced and electrogenic Cl secretion was evident. Na absorption was virtually abolished due to a significant decrease in JNaₘₜ and a significant increase in JNa₅,ₘₜ compared with control and day 3 infected values. Net Cl transport was reversed from a low rate of absorption to net secretion due to a significantly lower JCIₘₕ flux. Despite these differences in electrolyte fluxes in day 5 infected colon, PD and Isc were no different to control values due to the concommitant reduc-

### Table 2 Clinical and mucosal parameters in control animals, in EHEC infected animals, and in EHEC infected animals treated with the LTB₄ synthesis inhibitor MK-886

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Infected</th>
<th>Infected+MK-886</th>
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<tbody>
<tr>
<td>Weight gain (g)</td>
<td>66 (5)</td>
<td>59 (8)</td>
<td>34 (5)</td>
</tr>
<tr>
<td>Diarrhoea score</td>
<td>0</td>
<td>2.0 (0.3)</td>
<td>2.7 (0.1)</td>
</tr>
<tr>
<td>LTB₄ (pg/mg mucosa)</td>
<td>3.2 (0.7)</td>
<td>8.4 (1.4)*</td>
<td>2.0 (0.4)*</td>
</tr>
<tr>
<td>MPO activity (U/g mucosa)</td>
<td>2.9 (0.7)</td>
<td>33.9 (3.5)*</td>
<td>36.6 (7.4)*</td>
</tr>
<tr>
<td>Histology score</td>
<td>0.3 (0.1)</td>
<td>5.4 (0.3)*</td>
<td>5.3 (0.3)*</td>
</tr>
</tbody>
</table>

Data are mean (SEM) of n=9–24.

*p<0.05 compared with control; †p<0.05 compared with infected group.
MPO, myeloperoxidase.
tion in electrogenic Na absorption and development of electrogenic Cl secretion. Addition of indomethacin to the control distal colon caused a significant decrease in net Na absorption without any effect on Cl transport. The reduction in net Na absorption was due to a combination of significantly decreased $\Delta$Na$_{nm}$ and increased $\Delta$Na$_{cm}$. Control tissue PD, $I_{sc}$, and G were significantly reduced after exposure to indomethacin. Indomethacin had little impact on transport across day 3 infected tissue. It caused a small but significant decrease in net Na absorption as a result of an increase in $\Delta$Na$_{nm}$ but did not affect Cl transport, $P_{sc}$, or $I_{sc}$. In contrast, addition of indomethacin to day 5 infected tissue caused changes in both Na and Cl transport, significantly enhancing Na absorption and reversing Cl secretion to net absorption. The indomethacin-induced increase in Na absorption was due to a significant increase in $\Delta$Na$_{nm}$ above basal period values. Net Cl secretion was reversed to a low level of absorption after addition of indomethacin due to a significant fall in $\Delta$Cl$_{nm}$. Indomethacin also produced significant reductions in both PD and $I_{sc}$ in the day 5 infected group, consistent with impairment of an electrogenic ion transport process, most likely Cl secretion. However, indomethacin did not restore absorption of either Na or Cl to normal, with net fluxes of both electrolytes remaining significantly below control levels. After addition of indomethacin, net Na absorption remained lower in day 5 infected tissue than in control tissue because indomethacin had no effect on $\Delta$Na$_{cm}$ which remained significantly higher than the corresponding control value.

### Discussion

The aim of this study was to test current concepts on the pathophysiology of infectious colitis by assessing the roles of PGE$_2$ and LTB$_4$ in a reproducible well-characterised animal model. This also allowed us to further clarify the pathophysiology of EHEC colitis in infant rabbits and extend our previous time course observations in this model. We have reported in the past that inoculation of suckling rabbits with *E.coli* O157:H7 results in colonisation of the colon, mucosal inflammation, and diarrhoea. Chloride secretion develops on day 5 after inoculation while Na absorption is gradually impaired and eventually abolished. Histological injury, inflammation, and neutrophil infiltration also occur abruptly on day 5, coinciding with the onset of Cl secretion. A monoclonal antibody, which blocks neutrophil adhesion and emigration, prevents both the transport alterations and the histological damage, suggesting a pivotal role for neutrophils in the pathophysiology of EHEC infection.

A growing body of evidence suggests that eicosanoids contribute significantly to the pathogenesis of colitis. Indeed, an important secretagogue role has been ascribed to PGE$_2$ in current paradigms of the pathogenesis of infectious colitis. Available evidence also suggests that LTB$_4$ is a significant contributor to tissue inflammation and injury in experimental colitis. The current study assessed the roles of LTB$_4$ and PGE$_2$ in producing these end points in EHEC colitis. The results question the contribution of these eicosanoids in this model.

In the current study, LTB$_4$ levels increased markedly by day 2 after infection and remained high at later times. The levels did not correlate with the onset of mucosal inflammation (assessed histologically), damage (assessed by histology or by measurement of permeability), or neutrophil infiltration. Furthermore, successful inhibition of tissue LTB$_4$ synthesis with MK-886 failed to attenuate diarrhoea, mucosal neutrophil infiltration, or pathological inflammation and damage. (That the increase in LTB$_4$ synthesis failed to correlate with the development of ion transport changes was not surprising as LTB$_4$ has been shown not to affect colonic electrolyte transport.)

Closer correlations between the time courses of these parameters and LTB$_4$ levels might have been expected given the reported effects of LTB$_4$ on intestinal permeability and neutrophil chemotaxis and studies demonstrating that LTB$_4$ perpetuates tissue inflammation, injury, and ulceration in colitis. In trinitrobenzenesulphonic acid induced colitis, inhibitors of LTB$_4$ synthesis promoted healing of ulcerated mucosa and reduced the severity of inflammation and damage when administered before induction of colitis. Of note, however, are other reports that inhibition of LTB$_4$ synthesis does not attenuate human ulcerative
Presumably, enhanced synthesis of LT B4, on day 2 in the current study, occurring well before demonstrable tissue neutrophil infiltration (day 5), arises from other cells capable of leukotriene production, such as lamina propria fibroblasts or resident, rather than newly recruited, leukocytes. Resident neutrophils have been shown in another model of gut injury to contribute to mucosal dysfunction. Failure of elevated mucosal LT B4 levels to promptly attract neutrophils to the EHEC infected colonic mucosa suggests that other chemotactic factors may be responsible later in the evolution of the infection. Alternatively, some “permissive” factor or mediator, such as adhesion molecule expression on endothelial cells, may be absent until day 5 after EHEC inoculation when neutrophils eventually migrate into the colitic mucosa. Mucosal PGE2 levels also did not correlate well with changes in electrolyte transport. As PGE2 is a major paracrine regulator of fluid and electrolyte secretion in the colon, we anticipated that increases in mucosal PGE2 synthesis would be paralleled by the onset of Cl secretion and diminished Na absorption. Mucosal PGE2 synthetic capacity increased significantly by day 3 after infection but there were no changes in electrolyte transport at this time. By day 5, Cl secretion and impaired Na absorption were observed but indomethacin treatment only partially restored these abnormalities. The day 5 data (before and after indomethacin addition) would be consistent with the reported effects of exogenous PGE2, on colonic transport. The partial restoration of electrolyte absorption by indomethacin suggests that PGE2, or other cyclooxygenase products play some part in disrupting both Na and Cl transport in EHEC colitis. However, the lack of transport abnormalities on day 3 and the incomplete effect of indomethacin in reversing the day 5 changes suggest that additional factors contribute to transport disruption. For example, EHEC induced damage to absorptive surface epithelial colonocytes might account for failure of indomethacin to completely restore day 5 net Na and Cl absorption to control levels.

There are various possible explanations for these temporal discrepancies between changes in PGE2 levels and transport. Pro-absorptive inflammatory mediators, such as PGD2, interleukin-1β, or some neurotransmitters, might counteract the secretagogue effect of PGE2 at day 3. Synergism with another secretagogue might be necessary for the elevated levels of PGE2 to stimulate secretion, although numerous studies showing that PGE2 alone causes colonic Cl secretion make this a less likely explanation. Given our earlier finding that blockade of leucocyte adhesion and emigration prevented disruption of transport, we had wondered if neutrophil derived 5'-AMP might account for the abrupt onset of Cl secretion at day 5. Other possible explanations include alterations in the mucosal neurosecretory apparatus, alterations in prostaglandin E receptor subtype profile, and hyporesponsiveness of the inflamed colonic epithelium (as we and others have demonstrated in experimental colitis). Furthermore, we have recently demonstrated that within 18 hours of infection, EHEC renders T84 cells insensitive to Cl secretagogue stimulation.

Thus our results strongly suggest that the contribution of cyclooxygenase products to transport disruption is not as straightforward in EHEC colitis as in other experimental gut infections. This may be because of different experimental designs. In the cryptosporidiosis study mentioned above for example, transport and tissue PGE2 levels were measured in control tissue and at one time point after infection. Had we measured PGE2 synthesis and transport only in control and day 5 infected animals, then similar conclusions about the role of PGE2 might have been reached as PGE2 levels were substantially increased on day 5 (p=0.05 by unpaired t testing, day 5 infected vs control) and addition of indomethacin reversed transport changes, at least partially.

In conclusion, the present study provides further information on the pathophysiology of EHEC infection. Synthesis of eicosanoids is enhanced in the colonic mucosa of infected animals. While PGE2, or other cyclooxygenase products appear to account for at least some of the colonic transport abnormalities, the temporal relationship between increased mucosal PGE2 and Cl secretion is not as straightforward as previously assumed. Moreover, LT B4 did not appear to play a major role in mucosal inflammation and injury. Thus for both cyclooxygenase and lipoxygenase products, the current data imply a complicated interplay of factors in the pathogenesis of mucosal inflammation and electrolyte transport abnormalities during EHEC infection. This complexity may be an important means by which host defence mechanisms, such as intestinal secretion in response to luminal bacteria, are first upregulated and later switched off after elimination of the microbe.

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