NOTES

Roles of Leukotriene B₄, Prostaglandin E₂, and Cyclic AMP in Campylobacter jejuni-Induced Intestinal Fluid Secretion

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Infection of rabbit ileal loops with inflammatory Campylobacter jejuni strains caused elevation of cyclic AMP, prostaglandin E₂, and leukotriene B₄ levels in tissue and fluids. Incubation of cultured Caco-2 cells with loop fluids caused elevated cellular cyclic AMP levels, an effect which was inhibited by antisem against prostaglandin E₂.

Campylobacter jejuni enterocolitis in man is characterized by inflammatory infiltrate of neutrophils and mononuclear cells, villus degeneration and atrophy, loss of mucus, crypt abscess, and ulceration of mucosal epithelium (2, 9, 10, 13, 19, 22). In these respects, the disease is histologically similar to acute exacerbations of ulcerative colitis and indeed may be indistinguishable in the later stages when chronic inflammatory cells are present (19). Inflammation in ulcerative colitis and other inflammatory bowel diseases is mediated in part by leukotriene and prostaglandin release from leukocytes (3). Leukotriene B₄ (LTB₄), for example, is chemotactic for neutrophils and is important in the characteristic infiltration of these cells in the acute inflammatory response (3). Prostaglandin E₂ (PGE₂) enhances the chemotactic activity of LTB₄ (20). These compounds are also important physiological regulators of intestinal fluid and ion transport. Thus, PGE₂ acts by decreasing active sodium and chloride absorption and increasing fluid secretion in both the small intestine and the colon by activation of adenylate cyclase (15-18). Prostaglandins also increase the propulsive activity of the gut (1), and so may contribute to diarrhea by decreasing contact time of intestinal fluids with the absorptive surface. Since Campylobacter enterocolitis in man involves histopathological changes that closely resemble those in ulcerative colitis, we sought to determine the role of inflammatory mediators in C. jejuni-induced fluid secretion in the rabbit ileal loop model.

We previously reported the effects of experimental infection of rabbit ileal loops with C. jejuni L115, C119, O81, and P71 isolated from cases of human enterocolitis (5). Strains L115, C119, and O81 secrete small amounts of a cholera-like enterotoxin, detected by their effects both on Chinese hamster ovary cells and in enzyme-linked immunosorbent assays with GM1 ganglioside and antibodies against the B subunit of cholera toxin (CT). Strain P71 does not produce material active in these assays (5). Nevertheless, all four strains caused histological damage in rabbit ileal loops similar to that observed by endoscopy of the patients. Moreover, in all cases, biochemical analysis of accumulated loop fluids indicated a significant secretory component suggestive of adenylylcyclase activation in infected tissue (5). Consistent with this, cyclic AMP (cAMP) levels in tissue homogenates were elevated in C. jejuni-infected loops (P = 0.06, Student’s t test for paired data) compared with those in control loops in each animal; levels were comparable to those in loops treated with CT (Fig. 1A; P = 0.94, Student’s t test), although cholera-like enterotoxin was not detectable (5). Infection with mutant strain C. jejuni NCTC 12189, which failed to induce tissue damage or fluid secretion in the rabbit model (5), gave essentially no increase in tissue cAMP levels (Fig. 1A) despite the fact that it secretes low levels of cholera-like enterotoxin (as judged by the enzyme-linked immunosorbent assay mentioned above). Mean levels of cAMP in colitis- and NCTC 12189-infected loop tissues were significantly different (P = 0.04, Student’s t test). Cyclic GMP levels in homogenized loop tissues were very low in infected loops and not significantly different from those of control loops (data not shown).

To determine the involvement of inflammatory mediators in C. jejuni pathogenesis, PGE₂ and LTB₄ were extracted from loop fluids as described previously (12) and quantified by using modified commercial radioimmunoassay kits. Consistent with the involvement of these compounds in leukocyte infiltration, statistically significant correlations (P = <0.001, Spearman ranked correlation) were observed between the levels of PGE₂ and LTB₄ and the numbers of polymorphs in loop fluids (Table 1). Fluid from loops treated with CT, by contrast, showed low LTB₄ and PGE₂ levels and no leukocyte infiltrate, reflecting the noninflammatory histological picture of cholera.

Levels of PGE₂ in loop tissue homogenates also were significantly elevated after infection with colitis strains of C. jejuni compared with those of uninfected loops in the same animal (Fig. 1B; P < 0.001, Student’s t test). There was,
however, no correlation between PGE$_2$ and cAMP levels in loop tissues. It may be that, at the time of animal sacrifice, cellular cAMP levels had decreased from peak levels required to induce pathological effects or that stimulated cells may have been shed from the infected mucosa. Moreover, the PGE$_2$ levels measured here may be in excess of those required simply to saturate available receptors. In contrast with infection with colitis strains, treatment with CT or infection with NCTC 12189 did not result in elevation of tissue PGE$_2$ levels (Fig. 1B; colitis versus CT, $P = 0.05$; colitis versus NCTC 12189, $P = 0.002$ [Student’s t test]).

The biological activity of PGE$_2$ in secreted loop fluids was confirmed by determining their ability to elevate cAMP levels in monolayers of the human intestinal cell line Caco-2 (11), grown as described previously (4). Levels of cAMP were as much as 25-fold higher in cells treated with filter-sterilized loop fluids than in uninfected cells, compared with a 16-fold increase upon treatment with PGE$_2$ and a 400-fold increase upon treatment with CT (Fig. 2). Only minor increases in cAMP were observed in Caco-2 cells treated with broth-grown inflammatory strains of C. jejuni or with bacterial culture supernatants, perhaps because of the low level of cholera-like enterotoxin produced by these strains (4). However, loop fluids contained no detectable cholera-like enterotoxin (5), and enhancement of cAMP levels in fluid-treated Caco-2 cells was not inhibited by antiserum raised against the B subunit of CT; on the other hand, activity was reduced by antiserum raised against PGE$_2$ (Fig. 2).

Prostaglandin release from inflammatory cells has been

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**TABLE 1.** White cell infiltrate, LTB$_4$, and PGE$_2$ levels in rabbit ileal loop fluids infected with strains of *C. jejuni* from human colitis

<table>
<thead>
<tr>
<th>Treatment (no. of loops)</th>
<th>Polymorphs$^a$</th>
<th>PGE$_2$ (ng/ml)$^c$</th>
<th>LTB$_4$ (ng/ml)$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>L115 (4)</td>
<td>+/++</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>C119 (4)</td>
<td>+</td>
<td>1.7</td>
<td>0.34</td>
</tr>
<tr>
<td>O81 (3)</td>
<td>+/++</td>
<td>5.2</td>
<td>4.5</td>
</tr>
<tr>
<td>P71 (1)</td>
<td>+</td>
<td>5.2</td>
<td>5.0</td>
</tr>
<tr>
<td>CT (7)</td>
<td>−</td>
<td>0.63</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

$^a$ All values are averages of duplicate assays of each of the loop fluids recovered.

$^b$ Leukocytes per high-power field: +++, >20; ++, 10 to 20; +, 5 to 10; −, none.

$^c$ PGE$_2$ levels were measured with a modified commercial radioimmunoassay system (Amersham; sensitivity, approximately 20 pg/ml; specificity for prostaglandin E$_1$, 51%; specificity for prostaglandin F$_2\alpha$, 34%; less specificity with other eicosanoids).

$^d$ LTB$_4$ levels were measured with a modified commercial radioimmunoassay system (Amersham; sensitivity, 50 pg/ml; specificity, 20-OH LTB$_4$, 3.9%; less specificity with other eicosanoids).
proposed as a mechanism of fluid secretion in infectious inflammatory diarrhea (6–8, 21). Thus, indomethacin, an inhibitor of prostaglandin synthesis, abolished fluid accumulation in rabbit ileal loops infected with Salmonella spp. and reduced secretion due to Shigella flexneri (7, 8). Moreover, ileal secretion induced by Salmonella spp. was abolished in the absence of infiltration by leukocytes (7, 23), themselves potent sources of prostaglandins, leukotrienes, and other inflammatory mediators. However, activation of adenylate cyclase has not been detected in colonic inflammation associated with shigellosis and salmonellosis in humans (3). The work reported here relates elevated tissue cAMP levels with the host inflammatory mediator PGE₂ in rabbit ileal loops infected with C. jejuni and suggests a mechanism similar to that proposed for inflammatory bowel diseases (14), in which active secretion is stimulated in acute and chronic inflammation of the intestine.

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