INFECTION AND IMMUNITY, Nov. 1993, p. 4885–4887
0019-9567/93/114885-03$02.00/0

Vol. 61, No. 11

NOTES

Roles of Leukotriene B_4, Prostaglandin E_2, and Cyclic AMP in *Campylobacter jejuni*-Induced Intestinal Fluid Secretion

P. H. EVEREST,[1]† A. T. COLE,[2] C. J. HAWKEY,[2] S. KNUTTON,[3] H. GOOSSENS,[4]‡ J.-P. BUTZLER,[4] J. M. KETLEY,[1] AND P. H. WILLIAMS[1]*

Department of Genetics, University of Leicester, Leicester LE1 7RH,[1] Department of Therapeutics, University Hospital, Nottingham NG7 2UH,[2] Institute of Child Health, University of Birmingham, Birmingham B16 8ET,[3] United Kingdom, and WHO Reference Centre for Enteric Campylobacter, St. Pieters University Hospital, B-1000 Brussels, Belgium[4]

Received 1 March 1993/Returned for modification 16 April 1993/Accepted 10 August 1993

Infection of rabbit ileal loops with inflammatory *Campylobacter jejuni* strains caused elevation of cyclic AMP, prostaglandin E_2, and leukotriene B_4 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 antiserum against prostaglandin E_2.

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 B_4 (LTB_4), for example, is chemotactic for neutrophils and is important in the characteristic infiltration of these cells in the acute inflammatory response (3). Prostaglandin E_2 (PGE_2) enhances the chemotactic activity of LTB_4 (20). These compounds are also important physiological regulators of intestinal fluid and ion transport. Thus, PGE_2 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 adenylate cyclase 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, PGE_2 and LTB_4 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 PGE_2 and LTB_4 and the numbers of polymorphs in loop fluids (Table 1). Fluid from loops treated with CT, by contrast, showed low LTB_4 and PGE_2 levels and no leukocyte infiltrate, reflecting the noninflammatory histological picture of cholera.

Levels of PGE_2 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,

* Corresponding author.
† Present address: Department of Biochemistry, Imperial College, London SW7 2BB, United Kingdom.
‡ Present address: Laboratorium voor Mikrobiologie, Universitair Ziekenhuis Antwerpen, B-2650 Edegem (Antwerpen), Belgium.

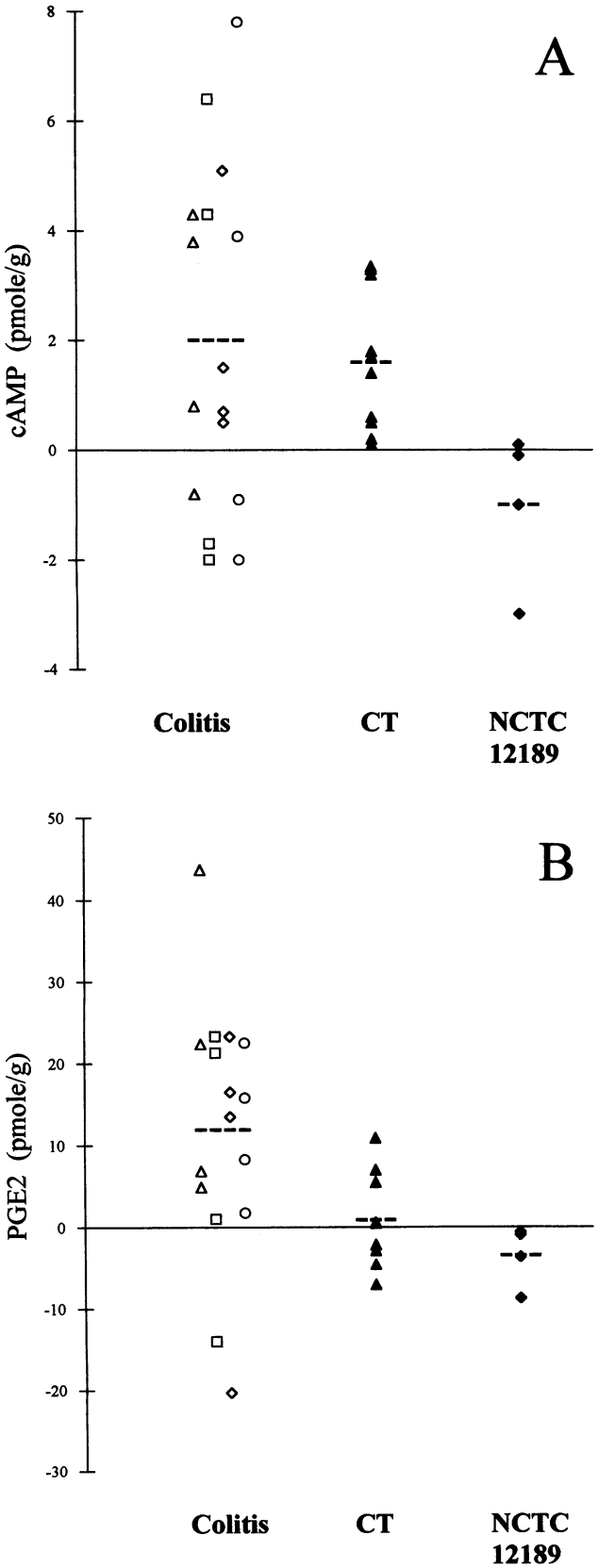

TABLE 1. White cell infiltrate, LTB_4, and PGE_2 levels in rabbit ileal loop fluids infected with strains of *C. jejuni* from human colitis[a]

Treatment (no. of loops)	Polymorphs[b]	PGE_2 (ng/ml)[c]	LTB_4 (ng/ml)[d]
L115 (4)	+/++	2.5	1.5
C119 (4)	+	1.7	0.34
O81 (3)	+++	5.2	4.5
P71 (1)	++	5.2	5.0
CT (7)	−	0.63	<0.05

[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 $F_{2\alpha}$ 3.4%; 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).

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

FIG. 1. Elevation of cAMP and PGE_2 in rabbit ileal loop tissue infected with *C. jejuni* L115 (○), C119 (△), O81 (□), and P71 (◇) from human colitis or with mutant strain NCTC 12189. Data for loops treated with CT are included for comparison. Each point, derived from duplicate assays, represents the difference in cAMP (A) or PGE_2 (B) concentrations between individual treated loops and untreated control loops in the same animal. cAMP levels were measured by using a commercial radioimmunoassay system (Amersham; sensitivity, approximately 320 pg/ml; specificity for cyclic GMP, <0.5%); PGE_2 was measured as described in Table 1, footnote *c*. Horizontal dashed lines indicate mean values for each group.

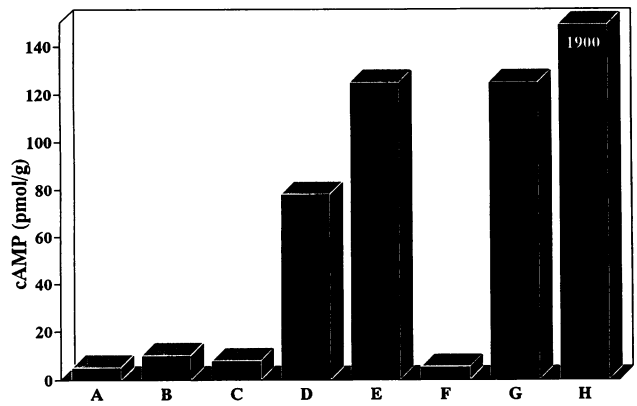

FIG. 2. PGE_2-induced elevation of cellular cAMP in Caco-2 cell monolayers treated with rabbit ileal loop fluids. Histograms represent cAMP concentrations in ethanol extracts of homogenized cells treated as described below and determined as described in the legend to Fig. 1. Bars: A, Uninfected; B, infected with strain C119 (10^7 cells per ml, 8 h); C, treated with overnight culture supernatant of strain C119 (8 h); D, treated with PGE_2 (10 ng/ml, 8 h); E, treated with filter-sterilized fluid from a rabbit ileal loop infected with strain C119 (4 h); F, treated as described for bar E but with added anti-PGE_2 antiserum; G, treated as described for bar E but with added anti-CT antiserum; H, treated with CT (2 μg/ml, 4 h). All values are means of at least three independent determinations.

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 PGE_2 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.

We acknowledge financial support from the Commission of the European Communities (Science Programme Twinning Grant to S.K., H.G., and P.H.W.), the Royal Society (J.K.), the Wellcome Trust (J.K.), and the Science and Engineering Research Council (studentship to P.E.).

We thank T. Wallis for help with histology.

REFERENCES

1. **Chang, E. B., and R. N. Fedorak.** 1985. Prostaglandins in diarrhoeal disease. J. Pediatr. Gastroenterol. Nutr. **4**:341–347.
2. **Colgan, T., J. R. Lambert, A. Newman, and S. C. Luk.** 1980. *Campylobacter jejuni* enterocolitis—a clinicopathologic study. Arch. Pathol. Lab. Med. **104**:571–574.
3. **Donowitz, M.** 1985. Arachidonic acid metabolites and their role in inflammatory bowel disease. Gastroenterology **88**:580–587.
4. **Everest, P. H., H. Goossens, J.-P. Butzler, S. Knutton, J. M. Ketley, and P. H. Williams.** 1992. Differentiated Caco-2 cells as a model for enteric invasion by *Campylobacter jejuni* and *C. coli*. J. Med. Microbiol. **37**:319–325.
5. **Everest, P. H., H. Goossens, P. Sibbons, D. R. Lloyd, S. Knutton, R. Leece, J. M. Ketley, and P. H. Williams.** 1993. Pathological changes in the rabbit ileal loop model caused by *Campylobacter jejuni* from human colitis. J. Med. Microbiol. **38**:316–321.
6. **Gianella, R. A.** 1979. Importance of the intestinal inflammatory reaction in salmonella-mediated intestinal secretion. Infect. Immun. **23**:140–145.
7. **Gianella, R. A., R. E. Gots, A. N. Charney, W. B. Greenough III, and S. B. Formal.** 1975. Pathogenesis of Salmonella-mediated intestinal fluid secretion: activation of adenylate cyclase and inhibition by indomethacin. Gastroenterology **69**:1238–1245.
8. **Gots, R. E., S. B. Formal, and R. A. Gianella.** 1974. Indomethacin inhibition of *Salmonella typhimurium*, *Shigella flexneri*, and cholera-mediated rabbit ileal secretion. J. Infect. Dis. **130**:280–283.
9. **Lambert, M. E., P. F. Schofield, A. G. Ironside, and B. K. Mandal.** 1979. Campylobacter colitis. Br. Med. J. **1**:857–859.
10. **Mee, A. S., M. Shield, and M. Burke.** 1985. Campylobacter colitis: differentiation from inflammatory bowel disease. J. Roy. Soc. Med. **78**:217–222.
11. **Pinto, M., S. Robine-Leon, M.-D. Appay, M. Kedinger, N. Triadou, E. Dussaulx, B. Lacroix, P. Simon-Assmann, K. Haffen, J. Foch, and A. Zweibaum.** 1983. Enterocyte-like differentiation and polarisation of the human colon carcinoma cell line Caco-2 in culture. Biol. Cell **47**:323–330.
12. **Powell, W. S.** 1988. High pressure liquid chromatography in the analysis of arachidonic acid metabolites, p. 75–98. *In* C. Benedetto, R. G. Mcdonald-Gibson, and S. Nigam (ed.), Prostaglandins and related substances—a practical approach. Oxford University Press, Oxford.
13. **Price, A. B., J. Jewkes, and P. J. Sanderson.** 1979. Acute diarrhoea: campylobacter colitis and the role of rectal biopsy. J. Clin. Pathol. **32**:990–996.
14. **Rachmilewitz, D., F. Karmeli, and Z. Selinger.** 1983. Increased colonic adenylate cyclase activity in active ulcerative colitis. Gastroenterology **85**:12–26.
15. **Racusen, L. C., and H. J. Binder.** 1980. Effect of prostaglandin on ion transport across isolated colonic mucosa. Digest. Dis. Sci. **25**:900–904.
16. **Rampton, D. S., and C. J. Hawkey.** 1984. Prostaglandins and ulcerative colitis. Gut **25**:1399–1413.
17. **Rask-Madsen, J.** 1986. Eicosanoids and their role in the pathogenesis of diarrhoeal diseases. Clin. Gastroenterol. **15**:545–566.
18. **Rask-Madsen, J., and K. Bukhave.** 1981. The role of prostaglandins in diarrhea, p. 58–70. *In* N. W. Read (ed.), Diarrhea: new insights. Janssen Pharmaceutical Ltd., London.
19. **Skirrow, M.** 1986. Campylobacter infections of man, p. 105–141. *In* C. S. F. Easmon (ed.), Medical microbiology, vol. 4. Academic Press, Inc., New York.
20. **Stenson, W. F.** 1990. Eicosanoids in inflammatory bowel disease with special reference to leukotriene B4, p. 273–281. *In* T. J. Peters (ed.), Cell biology of inflammation in the gastrointestinal tract. Corners Publications, London.
21. **Stephen, J., T. S. Wallis, W. G. Starkey, D. C. A. Candy, M. P. Osborne, and S. Haddon.** 1985. Salmonellosis: in retrospect and prospect. CIBA Found. Symp. **112**:175–192.
22. **Van Spreeuwel, P., G. C. Duursma, C. J. L. M. Meijer, R. Bax, P. C. M. Rosekrans, and J. Lindeman.** 1985. Campylobacter colitis: histological immunohistochemical and ultrastructural findings. Gut **26**:945–951.
23. **Wallis, T. S., R. J. H. Hawker, D. C. A. Candy, G.-M. Qi, G. J. Clarke, K. J. Worton, M. P. Osborne, and J. Stephen.** 1989. Quantification of the leukocyte influx into rabbit ileal loops induced by strains of *Salmonella typhimurium* of different virulence. J. Med. Microbiol. **30**:149–156.