Role of Intestinal Epithelial Cells in the Host Secretory Response to Infection by Invasive Bacteria

Bacterial Entry Induces Epithelial Prostaglandin H Synthase-2 Expression and Prostaglandin E_2 and $F_{2\alpha}$ Production

Lars Eckmann,* William F. Stenson,‡ Tor C. Savidge,§ David C. Lowe,§ Kim E. Barrett,* Joshua Fierer,* Jennifer R. Smith,* and Martin F. Kagnoff*

**Department of Medicine, University of California, San Diego, La Jolla, California 92093-0623; ‡Division of Gastroenterology, Department of Internal Medicine, Washington University, St. Louis, Missouri 63110; and §Department of Cellular Physiology, The Babraham Institute, Babraham, Cambridge CB2 4AT, United Kingdom*

Abstract

Increased intestinal fluid secretion is a protective host response after enteric infection with invasive bacteria that is initiated within hours after infection, and is mediated by prostaglandin H synthase (PGHS) products in animal models of infection. Intestinal epithelial cells are the first host cells to become infected with invasive bacteria, which enter and pass through these cells to initiate mucosal, and ultimately systemic, infection. The present studies characterized the role of intestinal epithelial cells in the host secretory response after infection with invasive bacteria. Infection of cultured human intestinal epithelial cell lines with invasive bacteria, but not noninvasive bacteria, is shown to induce the expression of one of the rate-limiting enzymes for prostaglandin formation, PGHS-2, and the production of PGE_2 and $PGF_{2\alpha}$. Furthermore, increased PGHS-2 expression was observed in intestinal epithelial cells in vivo after infection with invasive bacteria, using a human intestinal xenograft model in SCID mice. In support of the physiologic importance of epithelial PGHS-2 expression, supernatants from bacteria-infected intestinal epithelial cells were shown to increase chloride secretion in an in vitro model using polarized epithelial cells, and this activity was accounted for by PGE_2. These studies define a novel autocrine/paracrine function of mediators produced by intestinal epithelial cells in the rapid induction of increased fluid secretion in response to intestinal infection with invasive bacteria. (*J. Clin. Invest.* 1997. 100:296–309.) Key words: cyclooxygenase • inflammation • *Salmonella* • diarrhea • pathogenesis

Introduction

Pathogenic enteric bacteria, such as *Salmonella* and *Shigella*, are a significant health problem worldwide. Approximately 1% of the population in the United States is estimated to be infected with *Salmonella* annually (1). Infections with this pathogen are the most frequent cause of food-borne outbreaks of gastroenteritis in adults and children in this country (2, 3), and additionally, can cause invasive infections, mostly in the very young and elderly, and in immunosuppressed individuals. In developing countries, diarrheal disease due to infection with a variety of pathogenic bacteria is common in children, and has a high mortality secondary to dehydration (4, 5).

Pathogenic bacteria cause diarrhea by multiple mechanisms. For example, bacteria such as *Vibrio cholerae* reside in the lumen of the small intestine and produce toxins that alter ion absorption and/or secretion (6, 7). Other bacteria, such as *Shigella* and enteroinvasive *Escherichia coli*, invade and destroy the colonic epithelium, leading to dysentery (8). *Salmonella* and *Yersinia enterocolitica* pass through the intestinal epithelium and invade the mucosa (9–11). The ability of these bacteria to invade epithelial cells is important for disease pathogenesis since invasion mutants of *Salmonella* do not cause disease after oral infection (12), although production of an enterotoxin by some strains of *Salmonella* may also play a role in inducing diarrhea (13, 14). Studies in rabbits have shown that the onset of diarrhea occurs rapidly (within 2–4 h) in response to infection with some invasive enteric bacteria such as *Salmonella* (15). These data suggest that early events associated with bacterial invasion are important for initiating diarrhea.

Prostaglandins are important regulators of gastrointestinal fluid secretion (16, 17). PGE_1 stimulates chloride secretion in polarized cultured epithelial cells (18), and PGE analogues induce diarrhea in vivo (19). Moreover, prostanoids are common mediators of diarrhea of different etiologies, including radiation-induced diarrhea (20), and diarrhea after bacterial infection (21, 22). Prostaglandins are formed from free arachidonic acid through the conversion of arachidonic acid to PGH, which is catalyzed by the enzyme prostaglandin H synthase (PGHS)[1] (17, 23). PGH is subsequently converted by specific synthases to PGE, PGF, thromboxanes, or prostacyclins. PGHS exists in two isoforms: constitutively expressed PGHS-1, and inducible PGHS-2 (23). These isoforms have both overlapping and unique functions in the host, as demonstrated in genetically deficient mice. Thus, mice lacking PGHS-2 manifested renal dysplasia, cardiac fibrosis, and infertility, and were prone to peritonitis (24, 25). In contrast, PGHS-1–deficient mice had reduced platelet aggregation and a decreased inflammatory

Address correspondence to Lars Eckmann, Laboratory of Mucosal Immunology, University of California, San Diego, Department of Medicine 0623D, 9500 Gilman Drive, La Jolla, CA 92093-0623. Phone: 619-534-0683; FAX: 619-534-5691; E-mail: leckmann@popmail.ucsd.edu

Received for publication 25 February 1997 and accepted in revised form 15 April 1997.

J. Clin. Invest.

0021-9738/97/07/0296/14 $2.00
Volume 100, Number 2, July 1997, 296–309

1. *Abbreviations used in this paper:* CM, conditioned media; I_{SC}, short-circuit current; PGHS, prostaglandin H synthase; RT, reverse transcription.

response to arachidonic acid (26). Neither PGHS-1– nor PGHS-2–deficient mice had overt gastrointestinal abnormalities (24–26). Whereas PGHS-1 is expressed constitutively in many cell types, PGHS-2 normally is expressed at low levels in many tissues (23). High-level expression, however, can be induced in macrophages, and in epithelial cells in the intestine, kidney, and lungs (23, 27, 28). Known stimulators of PGHS-2 expression in epithelial cells include IL-1, TNFα, TGFα, and EGF (23, 29, 30).

Intestinal epithelial cells form a tight barrier that prevents mucosal penetration by most luminal bacteria. Some pathogenic enteric bacteria, however, invade the epithelium, and gain access to the underlying mucosa. Intestinal epithelial cells respond to bacterial invasion with the production of a range of chemoattractant and proinflammatory cytokines (31–33), suggesting that these cells, which are the first host cells to be entered by invasive enteric bacteria, produce early signals for the activation of the host inflammatory response.

In the present studies, we have characterized the role of intestinal epithelial cells in the rapid fluid response that is observed after infection with invasive bacteria. We show that bacterial invasion of intestinal epithelial cells induces the expression of PGHS-2, and stimulates the increased release of PGE_2 and $PGF_{2\alpha}$. Moreover, we show that PGE_2, released by these cells, can cause increased chloride secretion in an in vitro model of polarized colonic epithelial cells.

Methods

Cell lines. The human colon adenocarcinoma cell line HT-29 (ATCC HTB 38) and the embryonic intestinal cell line I407 (ATCC CCL 6) were obtained from the American Type Culture Collection (Rockville, MD), and were grown in DME supplemented with 10% FCS, 2 mM glutamine, and 10 mM Hepes (growth medium). T_{84} human colonic epithelial cells were grown as described before, and were cultured on permeable supports for Ussing chamber experiments (34).

Bacteria, cytokines, and other reagents. The following bacteria were used in these studies: *Salmonella dublin* lane (31), *Salmonella typhimurium* (ATCC 14028), *Salmonella typhi* Ty2 *aroA aroC* (strain BRD691) (35), *Yersinia enterocolitica* 08 (31), *Shigella dysenteriae* (31), *Listeria monocytogenes* (serotype 4b, ATCC 19115), enteroinvasive *Escherichia coli* (serotype O29:NM, ATCC 43892), enterohemorrhagic *E. coli* (serotype O157:H7, ATCC 43894), enteropathogenic *E. coli* (serotype O111, ATCC 33780), *Streptococcus bovis* (ATCC 9809), and three nonpathogenic *E. coli* strains: DH5α (GIBCO BRL, Gaithersburg, MD), SC13 (36), and HB101 (GIBCO BRL). The recombinant human cytokines TNFα and IL-1α, and goat antihuman TNFα (IgG isotype), were obtained from R & D Systems (Minneapolis, MN). Normal goat IgG, monoclonal mouse IgG_1 (MOPC-31c), LPS from *E. coli* serotype O111, and indomethacin were obtained from Sigma Chemical Co. (St. Louis, MO). Monoclonal mouse anti-PGE_2 (37) was a gift from Dr. J.P. Portanova (G.D. Searle & Co., St. Louis, MO). PGE_2, $PGF_{2\alpha}$, NS-398, and valerylsalicylate were obtained from Cayman Chemical Co. (Ann Arbor, MI).

Infection protocol. Colonic epithelial cells were seeded into 6-well tissue culture plates (Costar Corp., Cambridge, MA), and grown to confluence. Bacteria were prepared for infections as described previously (31, 38). Bacteria were added in a 1-ml vol to each well, and incubated for 1 h to allow bacterial entry to occur. Monolayers were washed three times to remove extracellular bacteria, and the cultures were further incubated for up to 29 h in the presence of 50 μg/ml gentamicin to kill any remaining extracellular bacteria.

To assay prostaglandin production, cultures were washed three times with DME, and were incubated for 15 min at 37°C in 1 ml/well of DME, 10 mM Hepes, 2 mg/ml BSA, 50 μg/ml gentamicin, and 20 μM arachidonic acid. Supernatants were collected, centrifuged to remove debris, and stored at −80°C.

To test conditioned media from bacteria-infected epithelial cell cultures for their ability to induce prostaglandin production by uninfected cells, supernatants were removed from cultures infected with *S. dublin* or *Y. enterocolitica* for 8 h, filtered through a 0.22 μm filter, and kept frozen at −80°C. Increasing doses of the 8-h supernatants from bacteria-infected and control epithelial cultures were added to confluent HT-29 cell monolayers in 6-well plates, and cultures were incubated for 8 h before prostaglandin production was determined as described above.

Prostaglandin immunoassays, [^{3}H]arachidonic acid labeling, and HPLC analysis of labeled cell supernatants. Levels of PGE_2 and $PGF_{2\alpha}$ were determined in culture supernatants by enzyme immunoassay (Cayman Chemical Co.). To confirm the identity of the prostaglandins, confluent I407 or HT-29 monolayers, in 10-cm plates, were infected for 1 h with 2.5×10^8 *S. dublin*, washed, and incubated for 7 h with gentamicin. Subsequently, cultures were incubated for 15 min in a 2.5-ml vol of DME containing 10 mM Hepes, 2 mg/ml BSA, and 20 μM arachidonic acid, as well as 5 μCi or 50 μCi of [5,6,8,9,11,12,14,15-^{3}H(N)]arachidonic acid (100 Ci/mmol; NEN Life Science Products, Boston, MA) for I407 or HT-29 cells, respectively. Supernatants were extracted with chloroform, and the chloroform-soluble phase was dried under nitrogen, reconstituted in methanol, and analyzed by reverse-phase HPLC (model 1090M; Hewlett-Packard Co., Palo Alto, CA) using an analytical C-18 Microsorb column (4.6 × 100 mm, Rainin Instrument Co., Woburn, MA). A flow rate of 1.0 ml/min was used with two solvents (A and B) set at 33.5% B for 0–10 min, 58.6% B for 11–25 min, and 92.0% B for 26–33 min. Solvent A was 100:0.01 (vol/vol) water/acetic acid, and solvent B was 100:0.01 (vol/vol) acetonitrile/acetic acid. The eluate was monitored with a spectrophotometer set at 205 nm, and the outflow from the spectrophotometer was routed to a Flo-One-beta detector (Radiomatic Instruments and Chemical Co. Inc., Tampa, FL) for radioactivity measurements. Prostanoids were identified by their elution times relative to known standards. The prostaglandin recovery after chloroform extraction and HPLC analysis was ∼ 75%, as determined by parallel analysis of known amounts of [^{3}H]PGE_2.

RNA extraction and RNase protection assays. Total cellular RNA was extracted using an acid guanidinium-phenol-chloroform method (Trizol; GIBCO BRL). PGHS-2 mRNA levels were determined by RNase protection assay using a ^{32}P-labeled antisense RNA probe specific for human PGHS-2 (39). The probe was prepared by in vitro transcription of a pGEM5Z plasmid containing a PGHS-2 specific insert (a gift from K. Seibert, G.D. Searle & Co.) to yield a 400-nt product. Subsequently, 10–20 μg total RNA was mixed with 10^5 cpm of specific RNA probe in hybridization buffer (Ambion Inc., Austin, TX), and the mixtures were incubated overnight at 45°C. Reactions were digested with optimal concentrations of RNase A and T1, and run on a 6% polyacrylamide/urea gel. Gels were dried, exposed to x-ray film, and band intensities were quantitated with a densitometer (model GS-670; Bio-Rad Laboratories, Hercules, CA). Absolute PGHS-2 mRNA levels were derived from a standard curve that was established from the band intensities of reactions containing known amounts of in vitro–transcribed PGHS-2 sense RNA.

Reverse transcription (RT)-PCR analysis. Reverse transcription and PCR amplification were performed as described before (32). The following primers were used to amplify a 305-bp fragment of PGHS-2 from human cDNA: 5′-TTC AAA TGA GAT TGT GGG AAA ATT GCT-3′ (sense) and 5′-AGA TCA TCT CTG CCT GAG TAT CTT-3′ (antisense). The primer sequences are located on different exons of the human PGHS-2 gene. They are specific for human PGHS-2, and do not amplify a product from mouse RNA or DNA. The primers for amplification of human β-actin mRNA were described before (32). Annealing temperatures were 60°C for PGHS-2, and 72°C for β-actin.

The general strategy used for quantitative RT-PCR analysis using internal standard RNAs, and the construct for the quantitation of hu-

man β-actin mRNA were described before (32). For the quantitation of human PGHS-2 mRNA, a DNA fragment was constructed as follows: a 660-bp *Pvu*II fragment of a cDNA clone from the mouse immunoglobulin α constant region (40) was amplified with the primers 5′-TGA GAT TGT GGG AAA ATT GCT **CTA CAG TGT GTC CAG CGT CC**-3′ (sense) and 5′-TCA TCT CTG CCT GAG TAT CTT **GCA ACA CGC TTG TCA CCA GG**-3′ (antisense). The regions homologous to the α constant region are depicted in bold. The resulting 392-bp DNA fragment was reamplified with the primers 5′-ATT GTA ATA CGA CTC ACT ATA GGG TTC AAA TGA GAT TGT GGG AAA ATT GCT-3′ (sense) and 5′-TTT TTT TTT TTT TTT TTT GAC AGA TCA TCT CTG CCT GAG TAT CTT-3′ (antisense) to yield a 446-bp DNA fragment. The sense primer contains a promoter for T7 RNA polymerase (underlined), and the antisense primer a stretch of dT (underlined) to provide a poly(A) tail after in vitro transcription. The latter DNA fragment was column-purified (QIA quick-spin PCR purification Kit; QIAGEN Inc., Chatsworth, CA), and in vitro–transcribed using T7 RNA polymerase to yield a 425-nt RNA. The reaction was digested with RNase-free DNase, extracted with phenol/chloroform, precipitated, and resuspended in TE. The OD260 was determined, and the number of standard RNA molecules per microliter was calculated based on the RNA concentration and the size of the standard RNA. Dilutions of standard RNA were made in 10 mM Tris pH 7.6, 1 mM EDTA containing 1 mg/ml tRNA to avoid nonspecific loss of transcripts at low concentrations. Reverse transcription and PCR amplification of the standard RNA using the PGHS-2 primers described above yield a 401-bp DNA fragment.

Immunoblot analysis. Confluent epithelial monolayers in 6-well plates were washed with ice-cold PBS, and lysed in 0.5 ml/well lysis buffer (150 mM NaCl, 20 mM Tris, pH 7.5, 0.1% Triton X-100, 1 mM PMSF, 10 μg/ml aprotinin). Lysates were sonicated for 5 s on ice, and centrifuged at 12,000 *g* for 2× 20 min. Protein concentrations in the lysates were determined by the Bradford method (Bio-Rad Laboratories) using BSA as a standard. 15 μg protein/lane was size-fractionated on a denaturing, nonreducing 6% polyacrylamide minigel (Mini-PROTEAN II; Bio-Rad Laboratories), and electrophoretically transferred to a nitrocellulose membrane (0.1-μm pore size). Specific proteins were detected using optimal concentrations of rabbit antihuman PGHS-2 (COOH terminus) (Oxford Biomedical Research, Oxford, MI), rabbit antihuman PGHS-1 (Oxford Biomedical Research), or rabbit antiactin (Sigma Chemical Co.) as primary antibodies, and peroxidase-conjugated donkey anti–rabbit Ig (Amersham Corp., Arlington Heights, IL) as secondary antibody. Specifically bound peroxidase was detected by enhanced chemiluminescence (ECL system; Amersham Life Science) and exposure to x-ray film (XAR5; Eastman Kodak Company, Rochester, NY) for 10–30 s.

Immunocytochemistry. HT-29 cells were grown on 13-mm glass coverslips in 24-well plates, infected for 1 h with 10^8/well *S. typhi* BRD691 in a 1-ml vol, washed, and further incubated for 4 or 24 h with gentamicin. Cultures were washed with PBS, and fixed at room temperature with 2% formalin in PBS for 30 min. After further washing, cultures were incubated sequentially for 1 h each at room temperature with optimal concentrations of rabbit antihuman PGHS-2 or antihuman PGHS-1 (Oxford Biomedical Research), biotin-labeled donkey anti–rabbit IgG (Amersham), and streptavidin-FITC (Amersham). Coverslips were mounted on glass slides using an aqueous mounting medium. For colocalization experiments, cultures were first stained for PGHS-2, and were subsequently incubated with optimal concentrations of goat anti-*Salmonella* common structural antigen-1 (CSA-1; Kirkegaard & Perry Laboratories, Gaithersburg, MD), and TRITC-labeled rabbit anti–goat IgG (Sigma Chemical Co.). Specimens were analyzed by confocal microscopy (MRC BioRad 500; Bio-Rad Laboratories). Control and infected specimens were analyzed under identical conditions so that the relative computer-generated color intensities (see Fig. 5) would be representative of the actual staining intensities, but not of the fluorochromes used.

Human fetal intestinal xenografts. The human fetal intestinal xenograft model used in the present work has been described in detail previously (41). Briefly, human fetal small intestine (n = 6, gestational age 10–14 wk) was transplanted subcutaneously into C.B-17 severe-combined immunodeficient (SCID) mice. Xenografts were allowed to develop for 10 wk before use, at which time the epithelium and underlying mucosa are fully differentiated (41). Xenografts were infected with $\sim 5 \times 10^7$ bacteria in DME/F12 medium in a 100-μl vol injected intraluminally by subcutaneous injection. Controls were injected with 100 μl sterile medium. Xenograft tissue was removed 6 h after infection, extensively washed, embedded in OCT compound, and snap-frozen in isopentane. Frozen sections (8 μm) of xenograft intestine were prepared, fixed and stained by indirect immunofluorescence, as described above for HT-29 cells, and counterstained with 5 μg/ml propidium iodide. To extract RNA from xenografts, tissues were removed 6 h after infection, and mucosal scrapings were prepared and immediately frozen in liquid nitrogen. Scrapings were homogenized on ice in 2 ml Trizol (GIBCO BRL) using a Potter-Elvehjem tissue grinder, and total RNA was extracted. The xenograft studies were performed with full approval from the Cambridge Local Ethics Committee, and in accordance with the Home Office guidelines specified in the Polkinghorne Report (1989) (38, 41).

Ussing chamber experiments. To test conditioned media for their ability to induce chloride secretion by polarized intestinal epithelial cells, confluent HT-29 cell monolayers in 162-cm^2 flasks were infected for 1 h with 1.5×10^9 *S. dublin*/flask in a 15-ml vol, washed, and further incubated for 7 h with gentamicin. Cultures were washed 3× with DME, and incubated for 15 min at 37°C with 7.5 ml/flask of DME, 10 mM Hepes, 2 mg/ml BSA, 50 μg/ml gentamicin, and 100 μM arachidonic acid. Supernatants were collected and filtered through a 0.22-μm filter. Ussing chamber experiments using polarized T_{84} cell monolayers as the target cell were then performed as described before (34).

Results

Salmonella infection of the human intestinal epithelial cell lines I407 and HT-29 increases PGE_2 and $PGF_{2\alpha}$ production. Oral *Salmonella* infection of monkeys rapidly induces increased intestinal fluid secretion through an indomethacin-sensitive pathway (22), suggesting that products of PGHS pathways are involved in this host response. To determine whether intestinal epithelial cells (which are the first host cells that are infected by *Salmonella* after oral ingestion) are involved in this host response to the infection, we determined if any, and which, products of the PGHS pathways are formed after *Salmonella* infection of these cells.

The human intestinal epithelial cell line I407 was infected for 1 h with *S. dublin*, and was cultured for an additional 7 h in the presence of gentamicin. Subsequently, infected and control cells were incubated for 15 min with [^{3}H]arachidonic acid, and the radioactive products in the culture supernatants were analyzed by HPLC. Control I407 cells constitutively produced PGE_2 and $PGF_{2\alpha}$, with PGE_2 being ~ 10-fold more abundant than $PGF_{2\alpha}$ (Fig. 1, *top*). The production of both prostaglandins increased by greater than fourfold in response to *S. dublin* infection of I407 cells (Fig. 1, *middle*). The absence of additional peaks suggests that PGE_2 and $PGF_{2\alpha}$ were the only PGHS products produced by the cells, since this HPLC system allows the separation and quantitation of all known PGHS products. The PGHS inhibitor indomethacin completely blocked production of PGE_2 and $PGF_{2\alpha}$ in *S. dublin*–infected I407 cells (Fig. 1, *bottom*), confirming that the arachidonic-derived products detected in the HPLC analysis were formed through a PGHS-dependent pathway. Consistent with the

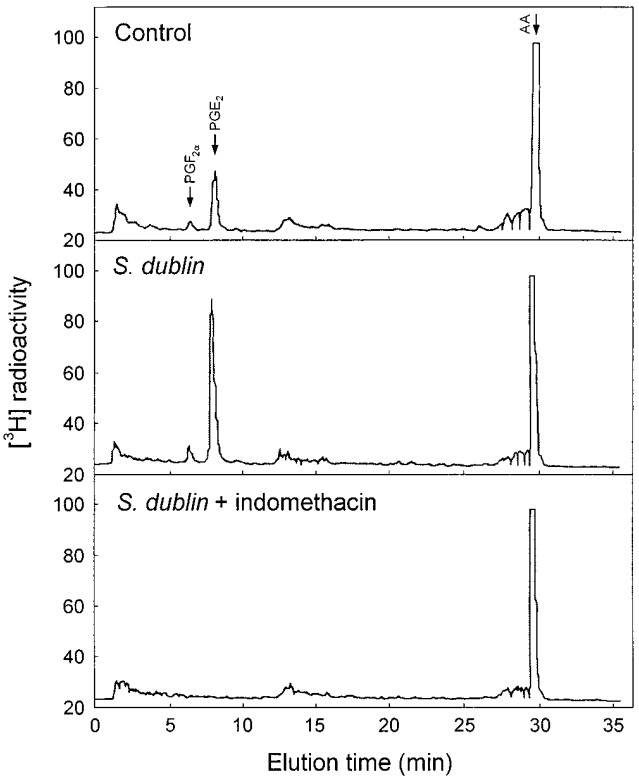

Figure 1. HPLC analysis of supernatants from [^{3}H]arachidonic acid–labeled I407 cells infected with *S. dublin.* Confluent I407 monolayers in 10-cm plates were infected with *S. dublin*, washed, and further incubated for 7 h with gentamicin. Cultures were washed, incubated for 15 min with 5 μCi [^{3}H]arachidonic acid, and supernatants were analyzed by HPLC using an on-line radioactivity detector as described in Methods. Uninfected cultures were used as a control. Indomethacin (3 μM) was added 1 h before the addition of [^{3}H]arachidonic acid, and was present during the 15-min incubation period with arachidonic acid. Peaks were identified by comparison with a range of prostaglandin standards. The *y*-axis reflects [^{3}H]radioactivity, measured as area under the curve of the radiochromatogram, with identical arbitrary units used in all three panels. In the middle panel (*S. dublin*), the PGE_2 and $PGF_{2\alpha}$ peaks represent 6 and 1% of the total recovered counts, respectively.

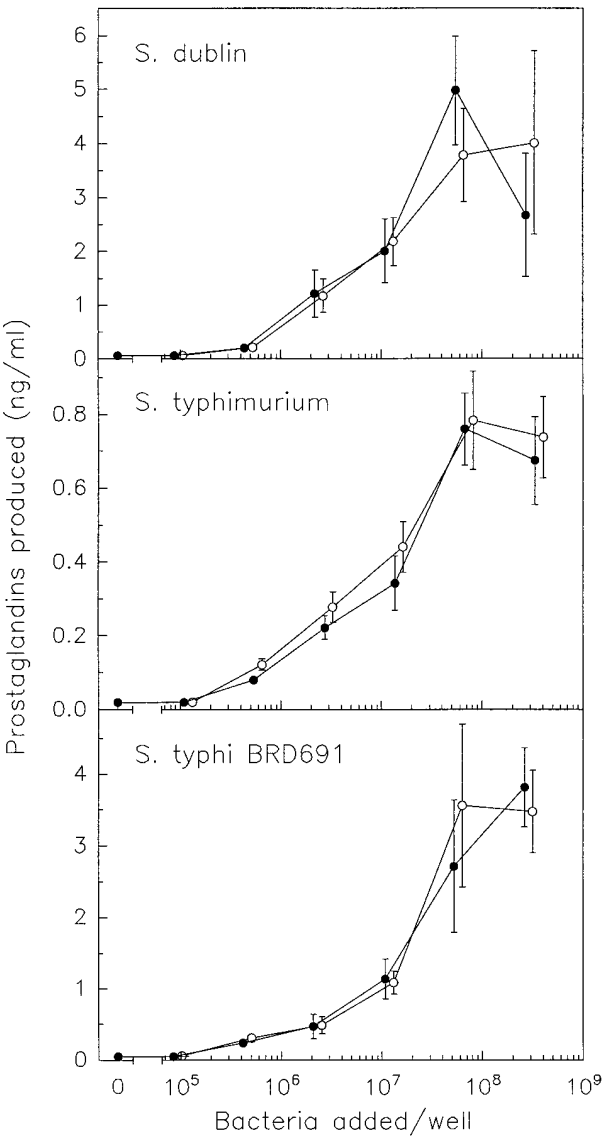

Figure 2. Relationship of bacterial inoculum and increased prostaglandin production in *Salmonella*-infected HT-29 cells. Confluent HT-29 monolayers in 6-well plates (containing $\sim 2 \times 10^6$ cells/well) were infected for 1 h with the indicated doses of *S. dublin* lane (*top*), *S. typhimurium* 14028s (*middle*), or *S. typhi* BRD691 (*bottom*), washed, and further incubated for 7 h with gentamicin. Cultures were incubated for 15 min in a 1-ml vol of DME containing 2 mg/ml BSA, and 20 μM arachidonic acid. PGE_2 (●) and $PGF_{2\alpha}$ (○) levels were analyzed by enzyme immunoassays. The identity of these prostaglandins was confirmed by HPLC analysis of supernatants from control and *S. dublin*–infected HT-29 cultures incubated for 15 min with [^{3}H]arachidonic acid (data not shown). Cultures infected with the three *Salmonella* strains contained similar numbers of intracellular bacteria at the end of the experiment, e.g., after infection with 10^8 CFU/well, monolayers had 1.8×10^7, 1.3×10^7, and 1.0×10^7 intracellular CFU/well for *S. dublin*, *S. typhimurium*, and *S. typhi* BRD691, respectively. Data represent means±SD of the results of triplicate cultures from one experiment. Similar results were obtained in at least two additional experiments for each bacterial strain.

data obtained from the HPLC analysis, *S. dublin*–infected I407 cells produced ninefold more PGE_2 than did control cells (73.3±23.1 vs. 8.4±2.6 ng/ml, values are means±SEM of three experiments) and fivefold more $PGF_{2\alpha}$ than control cells (6.7 vs. 1.3 ng/ml, means of two experiments), as determined by immunoassays of supernatants from *S. dublin*–infected and control I407 cultures that were incubated for 15 min with 20 μM arachidonic acid.

A second human intestinal epithelial cell line, HT-29, was used to confirm that the above findings were not restricted to a single cell line. As shown in Fig. 2, *S. dublin*–infected HT-29 cells produced up to 50-fold more PGE_2 and $PGF_{2\alpha}$ than controls during a 15-min incubation period with 20 μM arachidonic acid. In contrast to I407 cells, *S. dublin*–infected HT-29 cells produced similar levels of PGE_2 and $PGF_{2\alpha}$. Furthermore, two additional *Salmonella* strains, *S. typhimurium* and an invasive, but replication-deficient strain of *S. typhi* (strain BRD691), also induced increased PGE_2 and $PGF_{2\alpha}$ production by HT-29 cells, indicating that different *Salmonella* strains induce this response. For all three *Salmonella* strains, the increase in prostaglandin production was dependent on the bacterial inoculum, with maximal production at a bacterial inocu-

lum/cell ratio of ~ 50:1 (Fig. 2). Maximal prostaglandin production was fivefold lower after infection with *S. typhimurium* compared to *S. dublin* and *S. typhi*, although similar inoculum–response relationships were observed for all three *Salmonella* strains.

Increased PGE_2 and $PGF_{2\alpha}$ production by Salmonella-infected epithelial cells depends on increased PGHS-2 activity. Production of PGE_2 and $PGF_{2\alpha}$ depends on PGHS, which exists in two isoforms. To determine which PGHS isoform was important for increased prostaglandin production after *Salmonella* infection of epithelial cells, infected cells were preincubated for 60 min with several specific inhibitors before addition of 20 μM arachidonic acid. Incubation with indomethacin, an inhibitor of both PGHS-1 and PGHS-2, completely inhibited PGE_2 production in *S. dublin*–infected HT-29 cells (data not shown) and I407 cells (Fig. 1). In contrast, indomethacin did not affect increased IL-8 secretion by *S. dublin*–infected HT-29 cells, a previously reported response of these cells to infection with invasive bacteria (31), indicating that indomethacin had no general effects on the ability of these cells to respond to *Salmonella* (data not shown). Addition of NS-398, a specific PGHS-2 inhibitor (42), also completely inhibited increased PGE_2 production by *S. dublin*–infected HT-29 cells (*S. dublin*–infected, 22.6±2.7 ng PGE_2/ml; *S. dublin*–infected + 1 μM NS-398, < 0.2 ng/ml; controls, < 0.2 ng/ml; values are means±SD of triplicate cultures). Both indomethacin and NS-398 exhibited half-maximal inhibition of PGE_2 production after *S. dublin* infection of HT-29 cells at a concentration of ~ 30 nM. In contrast, valerylsalicylate, an inhibitor that is relatively specific for PGHS-1 (43), decreased PGE_2 production by *S. dublin*–infected HT-29 cells only slightly (*S. dublin*–infected, 22.6±2.7 ng PGE_2/ml; *S. dublin*–infected + 10 μM valerylsalicylate, 17.7±1.0 ng/ml; controls, < 0.2 ng/ml; values are means±SD of triplicate cultures). Thus, of the two PGHS isoforms, PGHS-2 is mostly, if not exclusively, responsible for increased PGE_2 production by HT-29 cells after *Salmonella* infection. This conclusion was also true for $PGF_{2\alpha}$ production, since indomethacin and NS-398 completely blocked increased $PGF_{2\alpha}$ production after *S. dublin* infection of HT-29 cells, whereas valerylsalicylate had no effect (data not shown).

Salmonella infection of intestinal epithelial cells upregulates protein and mRNA levels for PGHS-2, but not PGHS-1. Subsequent studies focused on mechanisms that govern epithelial expression of PGHS-2 in response to *Salmonella* infection. A time course of increased PGHS activity after infection of HT-29 cells with *S. dublin* is shown in Fig. 3 *A*. The ability of the cells to convert arachidonic acid to PGE_2 increased 3 h after *S. dublin* infection, reached maximal levels 12 h after infection, and remained increased until the end of the experiment (29 h). Similarly, $PGF_{2\alpha}$ production first increased 3 h after *S. dublin* infection of HT-29 cells, and reached a maximum 12 h after infection (controls, < 0.05 ng $PGF_{2\alpha}$/ml; 3 h after infection, 0.49±0.08 ng/ml; 12 h after infection, 10.7±1.0 ng/ml; values are means±SD of triplicate cultures).

To determine whether the increase in PGHS activity correlated with increased PGHS levels, lysates of *S. dublin*–infected and control HT-29 cells were analyzed by immunoblot analysis (Fig. 3 *B*). Increased levels of PGHS-2 were first detected 3 h after *S. dublin* infection, were maximal 6–10 h after infection, and remained increased thereafter. As a comparison, TNFα also stimulated increased PGHS-2 levels, and time course and maximal extent of the increase was similar to that after *S. dub-*

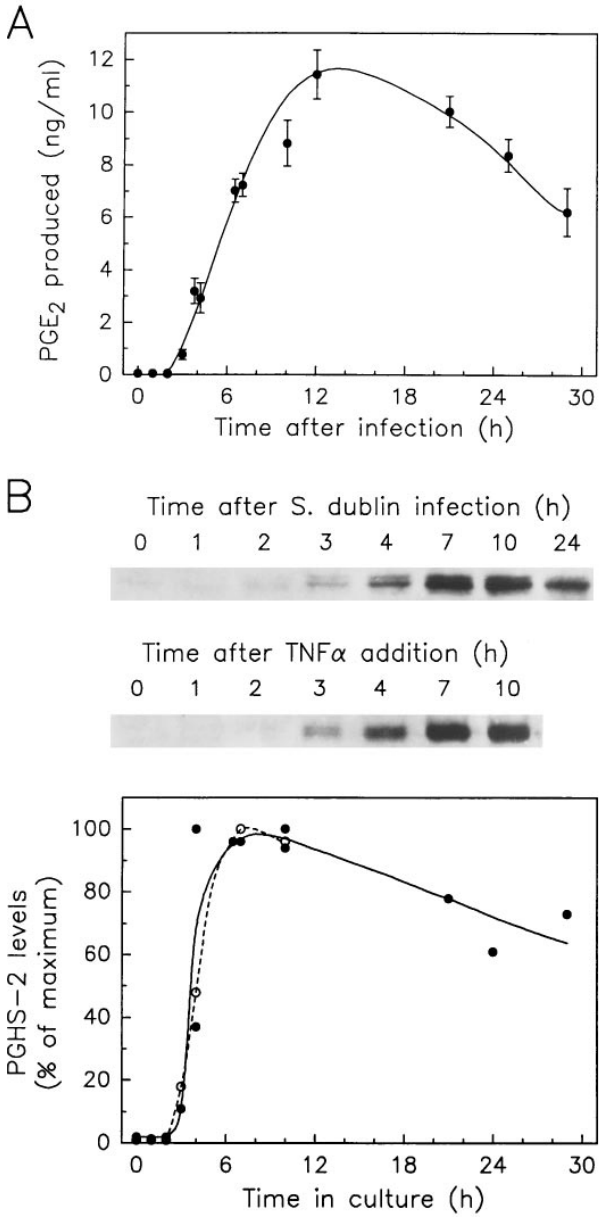

Figure 3. Time course of increased PGE_2 levels and PGHS-2 levels after *S. dublin* infection of HT-29 cells. Confluent monolayers of HT-29 cells in 6-well plates were infected for 1 h with 10^8 *S. dublin*/well, washed, and further incubated with gentamicin. (*A*) PGE_2 levels. At the indicated times after infection, cultures were washed, incubated for 15 min in a 1-ml vol of DME containing 2 mg/ml BSA, and 20 μM arachidonic acid, and PGE_2 levels were analyzed by enzyme immunoassay. Data represent means±SD of the results of triplicate cultures. Data shown are combined from two separate experiments. Parallel uninfected control cultures produced < 0.2 ng/ml PGE_2 at all time points. (*B*) PGHS-2 levels. At the indicated times after *S. dublin* infection or TNFα addition (20 ng/ml), cell lysates were prepared, size-fractionated, and blotted onto a nitrocellulose membrane. PGHS-2 was detected with a specific antibody and enhanced chemiluminescence. Representative examples of x-ray films are shown. To obtain quantitative data, x-ray films were scanned with a densitometer. PGHS-2 levels are expressed relative to the maximum levels in each experiment. Data from two separate experiments are depicted in the graph. ●, PGHS-2 levels after *S. dublin* infection; ○, PGHS-2 levels after TNFα stimulation.

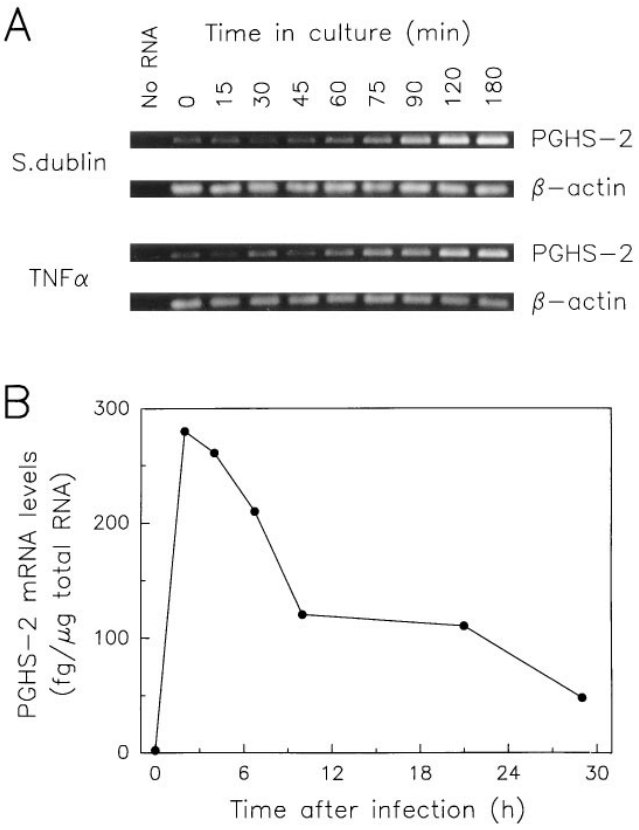

Figure 4. Kinetics of increased PGHS-2 mRNA expression in *S. dublin*–infected HT-29 cells. Confluent HT-29 monolayers in 6-well plates were infected for 1 h with 10^8 *S. dublin*/well, washed, and further incubated with gentamicin. Parallel cultures were stimulated with 20 ng/ml TNFα. Total cellular RNA was extracted at the indicated times after *S. dublin* infection or TNFα stimulation. Unstimulated, uninfected cultures were used as controls (designated as 0 min or 0 h in the figure). PGHS-2 mRNA levels were assessed by RT-PCR and RNase protection assays. (*A*) RT-PCR analysis. Total RNA (250 ng for PGHS-2, 100 ng for β-actin) was reverse-transcribed using an oligo(dT) primer, and amplified by PCR for 33 (PGHS-2) or 32 cycles (β-actin). One-fifth of the PCR reactions was run on a 1.2% agarose gel, which was stained with ethidium bromide and photographed using Polaroid 667 film. As a control, RNA was omitted from reverse transcription and PCR amplification (*No RNA*). (*B*) RNase protection assay. Levels of PGHS-2 mRNA were determined by RNase protection assays as described in Methods. Data points represent means of two experiments.

lin infection (Fig. 3 *B*). In contrast, levels of PGHS-1, as well the control protein actin, were not altered after *S. dublin* infection of HT-29 cells or after TNFα stimulation (PGHS-1 levels were 98, 97, and 81% of controls, and actin levels were 99, 91, and 81% of controls at 4, 10, and 24 h after *S. dublin* infection, respectively, as determined by immunoblot analysis and scanning with a densitometer; $n = 2$). Infection of I407 cells with *S. dublin* also increased PGHS-2 levels, and the time course and relative magnitude of the increase were similar to those in HT-29 cells (data not shown).

Increased PGHS-2 levels were paralleled by increased PGHS-2 mRNA levels in *S. dublin*–infected HT-29 cells, as demonstrated by qualitative RT-PCR and confirmed quantitatively by RNase protection assays (Fig. 4). PGHS-2 mRNA levels first increased 60 min after *S. dublin* infection (Fig. 4 *A*), reached a maximum 2–4 h after infection, and decreased thereafter (Fig. 4 *B*). Similarly, *S. dublin* infection increased PGHS-2 mRNA levels in I407 cells (2–4 h after *S. dublin* infection, 535 fg PGHS-2 mRNA/μg total RNA; controls, 92 fg/μg; values are means, $n = 2$). In summary, PGHS-2 mRNA levels increased rapidly, but transiently, after *S. dublin* infection of HT-29 and I407 cells, whereas the increase in PGHS-2 levels and PGHS activity occurred more slowly after infection, but was more sustained.

PGE_2 production by HT-29 colonic epithelial cells is increased in response to infection with invasive but not noninvasive bacteria. The above studies focused on the PGE_2 and $PGF_{2\alpha}$ response of cultured intestinal epithelial cells to infection with different *Salmonella* strains. To determine whether other bacteria can induce a similar epithelial response, HT-29 cells were infected with a range of invasive and noninvasive gram-negative and gram-positive bacteria, and PGE_2 production was determined. As shown in Table I, infection of HT-29 cell cultures with the invasive gram-negative bacteria *Y. enterocolitica*, *S. dysenteriae*, and enteroinvasive *E. coli* (serotype O29:NM) increased PGE_2 production 17–54-fold. In contrast, infection with several noninvasive gram-negative bacteria, including enteropathogenic and enterohemorrhagic *E. coli* strains, or addition of bacterial LPS, increased PGE_2 production by HT-29 cells to a much lesser extent (less than fivefold). Moreover, infection of HT-29 cell cultures with the gram-positive invasive bacteria *L. monocytogenes* also increased PGE_2 production, although the increase was lower than that seen after infection with gram-negative invasive bacteria. Infection with the noninvasive gram-positive *S. bovis* had little effect on PGE_2 production by HT-29 monolayers. Thus, invasive bacteria increase epithelial PGE_2 production, while noninvasive do not, or to a much lesser extent. In addition, levels of $PGF_{2\alpha}$ production paralleled those of PGE_2 in all cases tested (data not shown), further underlining that PGE_2 and $PGF_{2\alpha}$ production are likely regulated by identical mechanisms in bacteria-infected epithelial cells.

PGHS-2 is expressed mostly by bacteria-invaded epithelial cells early after infection, while at later times noninfected cells also express PGHS-2. To characterize the relationship between invaded bacteria and increased PGHS-2 expression, HT-29 cells were infected with a replication-deficient *S. typhi* (strain BRD691) and analyzed by double immunofluorescence for *Salmonella* staining and PGHS-2 expression (Fig. 5). Relatively early after infection (5 h), PGHS-2 expression was confined to a small proportion of cells, and was found mostly in cells which also contained *Salmonella*, suggesting that the induction of PGHS-2 resulted from a direct interaction between invading bacteria and infected cells. A small proportion of PGHS-2–expressing cells, however, did not appear to harbor bacteria, although these cells were located mostly in the immediate vicinity of *Salmonella*-infected cells.

At later times after infection (24 h) with *S. typhi* BRD 691, essentially all epithelial cells expressed PGHS-2 (Fig. 6 *B*). This observation was comparable to that in HT-29 cells stimulated with TNFα (Fig. 6 *C*), and both were in striking contrast to the findings in unstimulated cells (Fig. 6 *A*). Since the monolayers were infected with a replication-deficient strain of *S. typhi*, and gentamicin was present throughout the culture period, it is unlikely that bacterial replication or spread could have occurred in these cultures. This observation suggests that

Table I. Increased PGE_2 Production by HT-29 Colon Epithelial Cells in Response to Infection with Invasive Bacteria

Exp.	Additions to culture	Inoculum	Intracellular bacteria		PGE_2-produced	
			No./well	% of inoculum	ng/ml	Ratio infected/control
1	*S. dublin*	9.2×10^7	7.3×10^6	7.9	7.05±0.55*	54.2
	Y. enterocolitica	4.0×10^8	1.2×10^8	30.0	6.66±0.24	51.2
	S. dysenteriae	3.8×10^8	4.0×10^6	1.1	2.22±0.28	17.1
	None				0.13±0.01	1.0
2	*E. coli* O29:NM (enteroinvasive)	6.4×10^8	7.2×10^6	1.1	2.20±0.34	44.0
	E. coli O157 (enterohemorrhagic)	4.6×10^8	3.5×10^4	0.0076	0.12±0.01	2.4
	E. coli O111 (enteropathogenic)	3.3×10^8	1.0×10^4	0.0030	0.14±0.01	2.8
	E. coli DH5α (nonpathogenic)	2.4×10^8	3.6×10^3	0.0015	0.23±0.03	4.6
	E. coli HB101 (nonpathogenic)	3.2×10^8	1.4×10^3	0.00044	0.24±0.01	4.8
	LPS (10 μg/ml from *E. coli* O111)				0.09±0.01	1.8
	None				0.05±0.01	1.0
3	*L. monocytogenes*	7.5×10^7	1.9×10^7	25.3	1.07±0.07	7.6
	S. bovis	9.0×10^7	1.9×10^4	0.021	0.10±0.02	0.7
	None				0.14±0.02	1.0

Confluent HT-29 monolayers in 6-well plates were infected for 1 h with the indicated doses of bacteria, washed, and further incubated for 6 h with gentamicin. Uninfected cultures were used as controls. Cultures were washed and incubated for 15 min in a 1-ml vol of DME containing 2 mg/ml BSA, and 20 μM arachidonic acid. PGE_2 levels were analyzed by specific enzyme immunoassay. Monolayers were lysed in water, and Nos. of intracellular bacteria were determined by plating of serial dilutions on tryptic soy agar plates and overnight incubation. *Exp*., experiment. *Data represent mean±SD of the results from triplicate cultures.

the increased proportion of PGHS-2–expressing cells at later times after infection, in contrast to the early period after infection, did not result solely from a direct interaction between bacteria and infected cells, but was in part due to indirect stimulation of noninfected cells. In support of this notion, 8-h supernatants from *S. dublin*– or *Y. enterocolitica*–infected HT-29 cultures, but not from control cells, stimulated a > 30-fold increase in PGE_2 production by uninfected HT-29 monolayers (e.g., addition of 30% conditioned media from *S. dublin*–infected cells, 1.01±0.03 ng PGE_2/ml; from controls cells, < 0.03 ng/ml; values are means±SD of triplicate cultures).

Release of TNFα after Salmonella infection of intestinal epithelial cells is not important for mediating increased PGE_2 production. The studies above suggest that *Salmonella*-infected HT-29 cells release an activity that induces uninfected cells in the monolayer to express PGHS-2. Infection of colonic epithelial cell lines with invasive bacteria is known to induce the secretion of several proinflammatory cytokines, including IL-8 and TNFα (32). Since the latter is a potent stimulus for increased PGHS-2 expression (Fig. 3), we tested the possibility that TNFα release is important for mediating increased PGHS-2 expression in bacteria-infected intestinal epithelial cells.

As shown in Fig. 7 *A*, TNFα levels in the supernatants began to increase within 1 h after *S. dublin* infection of HT-29 cells, reached a maximum 2 h after infection, and remained increased thereafter. Maximal TNFα levels were dependent on the bacterial dose, and were as high as 125 pg/ml after *S. dublin* infection of HT-29 cells (Fig. 7 *A*). This TNFα level, however, was 5–10-fold lower than would be required (e.g., 0.5–1 ng/ml) to mediate the increase in PGE_2 production observed after *S. dublin* infection of HT-29 cells, as shown by a comparison of the dose–response curve for TNFα-induced PGE_2 production by HT-29 cells, with the PGE_2 response observed after *S. dublin* infection (Fig. 7 *B*). Furthermore, the dose–response relationship for TNFα-induced PGE_2 production was similar in uninfected and *S. dublin*–infected HT-29 cells, and no synergistic increase in PGE_2 production was observed in cells doubly stimulated with optimal numbers of *S. dublin* and optimal levels of TNFα, indicating that *S. dublin* infection neither sensitized the cells nor potentiated the cellular response to TNFα stimulation. Consistent with the interpretation that TNFα did not mediate the PGE_2 response to *Salmonella* infection, addition of anti-TNFα had no effect on *S. dublin*–induced PGE_2 production (Fig. 7 *C*). The identical concentration of anti-TNFα completely blocked the PGE_2 response to 5 ng/ml TNFα (Fig. 7 *C*).

In addition to TNFα, infection of HT-29 cells with invasive bacteria also increases IL-1β mRNA levels (32). IL-1β levels, however, were extremely low after *S. dublin* infection of HT-29 cells (< 3 pg/ml), and HT-29 cells do not express IL-1α mRNA nor contain IL-1α (32, 44), suggesting that neither IL-1α nor IL-1β were responsible for the increase in PGE_2 production after *Salmonella* infection.

Increased PGHS-2 expression by intestinal epithelial cells in vivo in a human intestinal xenograft model infected with Salmonella. We next asked whether PGHS-2 can also be induced in intestinal epithelial cells in vivo. To infect acutely human intestinal epithelial cells with invasive bacteria, we used a human fetal intestinal xenograft model where human fetal intestine (gestational age 10–14 wk) is transplanted subcutaneously into SCID mice. The xenografts develop a fully differentiated epithelial layer of entirely human origin over a 10–20-wk period (41). Fully differentiated xenografts were infected intraluminally with an invasive *S. typhi*, and PGHS-2 expression in the xenografts was analyzed by quantitative RT-PCR and immunofluorescence. As shown in Table II, human PGHS-2 mRNA levels increased > 40-fold after infection of the xenografts with

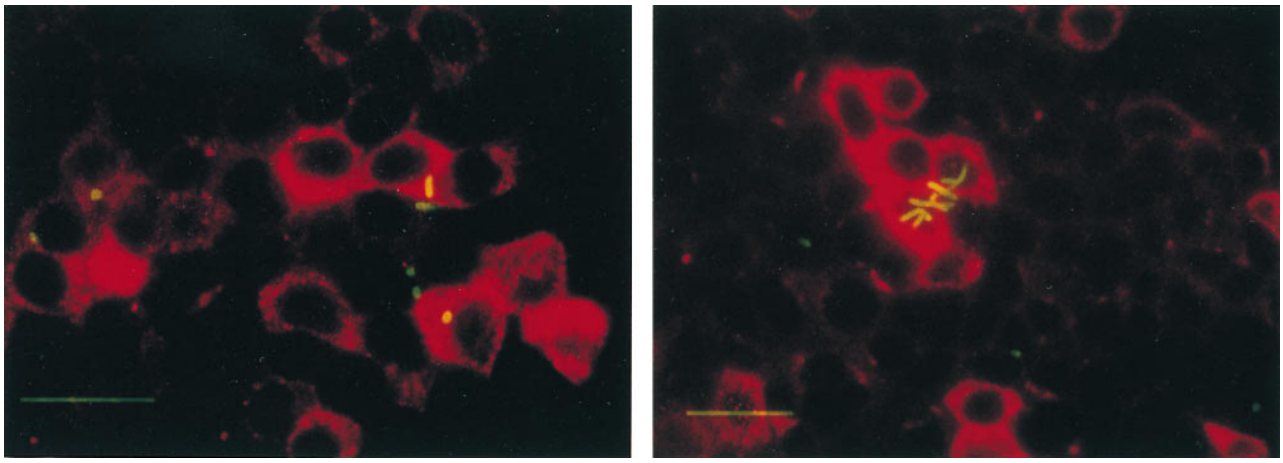

Figure 5. Colocalization of invaded *S. typhi* BRD691 and increased PGHS-2 expression early after infection of HT-29 epithelial cells. HT-29 monolayers on coverslips in 24-well plates were infected for 1 h with *S. typhi* BRD691, washed, and further incubated with gentamicin for 4 h. Cells were fixed, and stained by indirect immunofluorescence for PGHS-2 (*red*) and *Salmonella* common structural antigen-1 (*green*), as described in Methods. Overlapping of red PGHS-2 staining and green *Salmonella* staining results in a yellow color. As shown in two representative examples, PGHS-2 expression is mostly observed in cells which also contain *Salmonella.* In addition, a few cells express PGHS-2 but do not contain bacteria. These cells are located predominantly in the immediate vicinity of *Salmonella*-infected cells. The scale bars represent 25 μm. Staining for PGHS-1 yielded a weak signal, and PGHS-1 staining did not increase after *S. typhi* BRD691 infection (not shown).

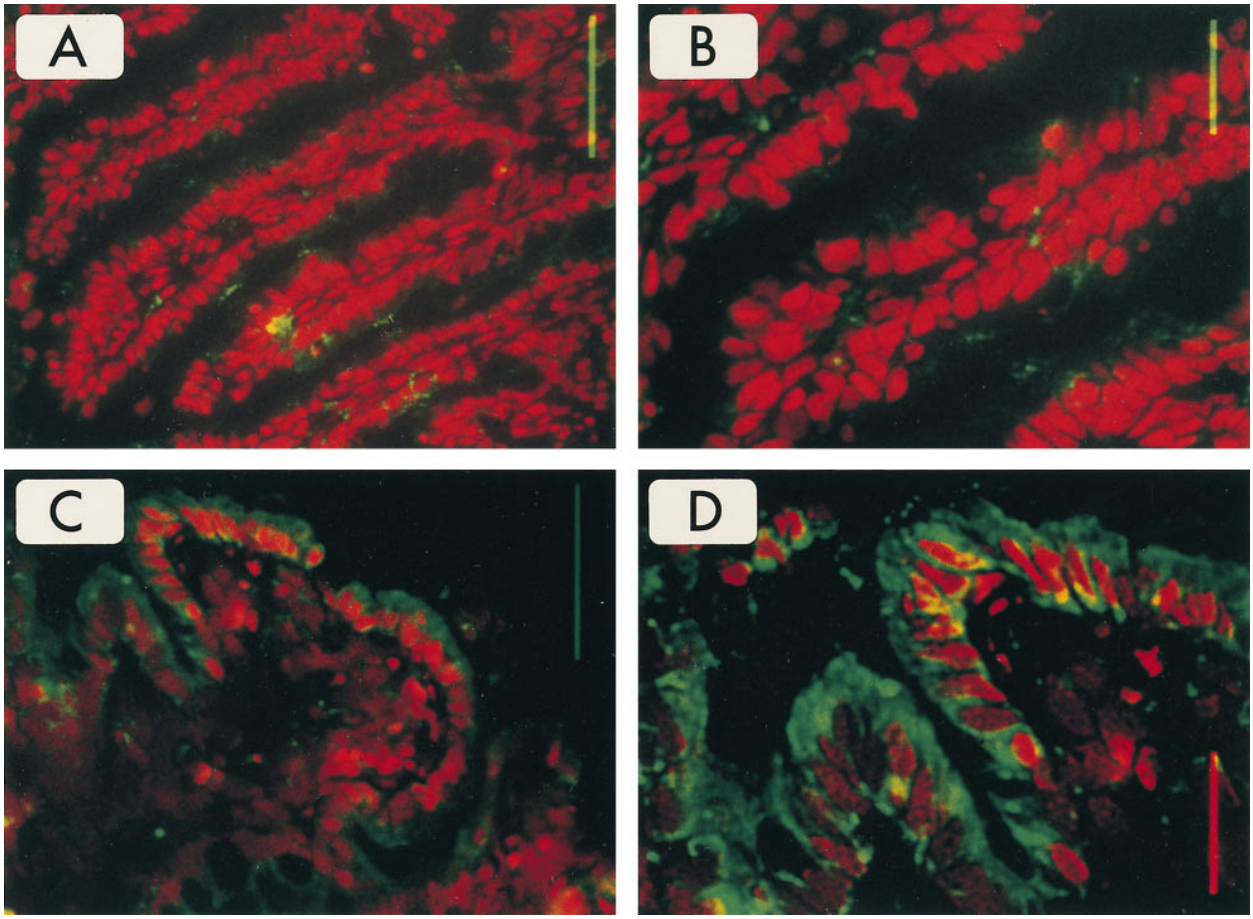

Figure 8. Salmonella infection induces increased PGHS-2 expression in intestinal epithelial cells in a human intestinal xenograft model. Mature human fetal intestinal xenografts in SCID mice were infected for 6 h with *S. typhi* BRD691, and 8-μm frozen sections were prepared as described in Methods. Sections were stained with rabbit anti–PGHS-2 (*green*) and counterstained with propidium iodide (*red*). *A* and *B* are sections from uninfected control xenografts at two different magnifications, and *C* and *D* are from *S. typhi*–infected xenografts at two different magnifications. The scale bars in *A* and *C* represent 25 μm, and those in *B* and *D* represent 10 μm. Staining with a control antibody (normal rabbit serum) yielded no specific staining (not shown).

Table II. Increased Expression of Human PGHS-2 mRNA in Bacteria-infected Human Fetal Intestinal Xenografts

Additions	PGHS-2		β-actin	
	No. of mRNA molecules/μg RNA	Ratio infected/control	No. of mRNA molecules/μg RNA	Ratio infected/control
S. typhi BRD691	1.2×10^5	44.4	1.2×10^7	0.9
S. typhi BRD691 (formalin-fixed)*	1.3×10^4	4.8	1.5×10^7	1.2
E coli SC13	9.2×10^3	3.4	1.4×10^7	1.1
None	2.7×10^3	1.0	1.3×10^7	1.0

Mature human fetal intestinal xenografts in SCID mice were infected with 5×10^7 bacteria in a 0.1-ml vol. Xenografts were removed 6 h later, total RNA was extracted from individual xenografts, and equal amounts of RNA were pooled from four to five mice for each group. Levels of the mRNAs for PGHS-2 and β-actin were determined by quantitative RT-PCR using internal RNA standards as described in Methods. *Bacteria were killed by incubation in 10% phosphate-buffered formalin (pH 7.2) for 30 min at room temperature. Killed bacteria were washed twice with PBS before injection into xenografts.

live *S. typhi*, whereas infection with killed *S. typhi*, or a live nonpathogenic *E. coli*, increased human PGHS-2 mRNA levels less than fivefold.

Because PGHS-2 expression was analyzed early after infection (6 h), at a time when *Salmonella* mostly reside inside epithelial cells, the RT-PCR data suggested that epithelial cells were mostly responsible for the increase in PGHS-2 mRNA expression in the xenografts. To document this directly, tissue sections were prepared and stained for PGHS-2 by indirect immunofluorescence. As shown in Fig. 8, *C* and *D*, intestinal epithelial cells stained positively for PGHS-2 after *S. typhi* infection of the xenograft, whereas little epithelial PGHS-2 staining was observed in uninfected control xenografts (Fig. 8, *A* and *B*). In contrast, staining for PGHS-1 showed weak constitutive

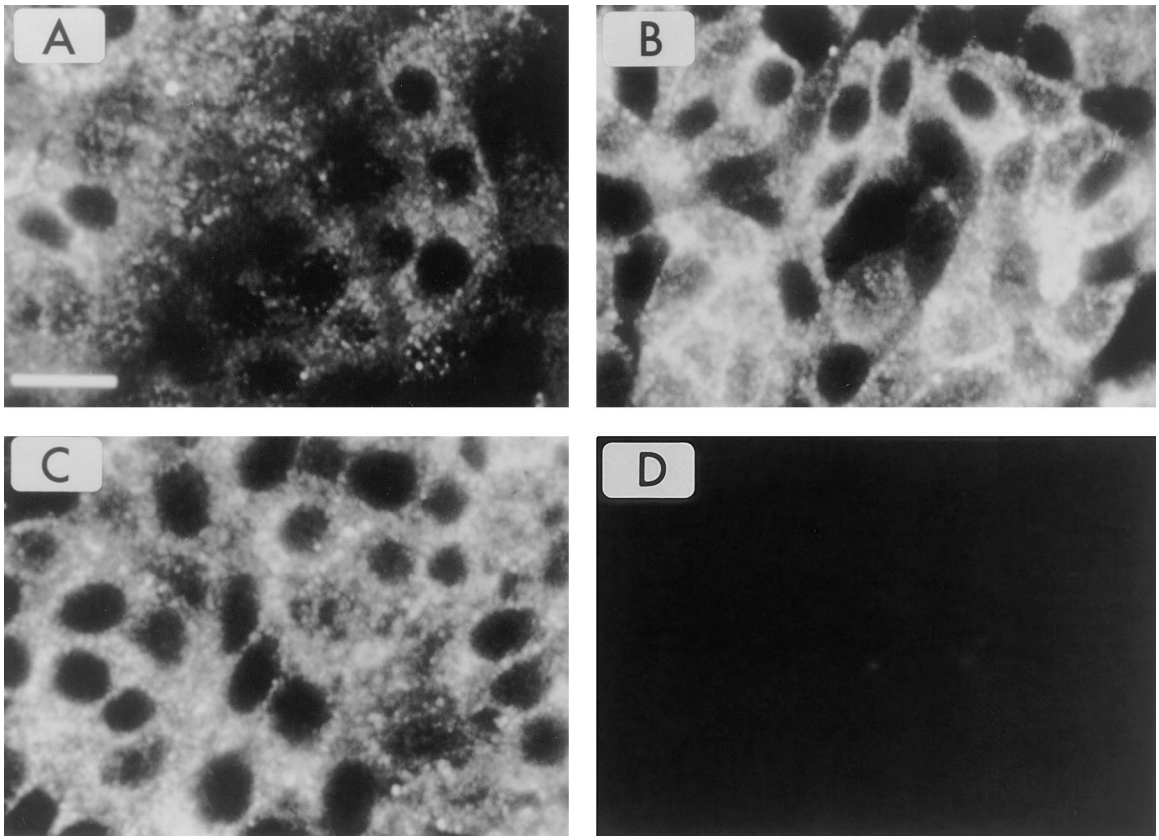

Figure 6. PGHS-2 expression in *S. typhi* BRD691–infected HT-29 cells 24 h after infection. HT-29 monolayers on coverslips were infected with *S. typhi* BRD691, and incubated with gentamicin for 24 h. Parallel cultures were stimulated for 24 h with 20 ng/ml TNFα. Cells were fixed, and stained by indirect immunofluorescence for PGHS-2. (*A*) Control cells stained with rabbit anti–PGHS-2; (*B*) *S. typhi*–infected cells stained with rabbit anti–PGHS-2; (*C*) TNFα-stimulated cells stained with rabbit anti–PGHS-2; and (*D*) *S. typhi*–infected cells stained with a control antibody (rabbit antihuman CD3). The scale bar in *A* represents 25 μm.

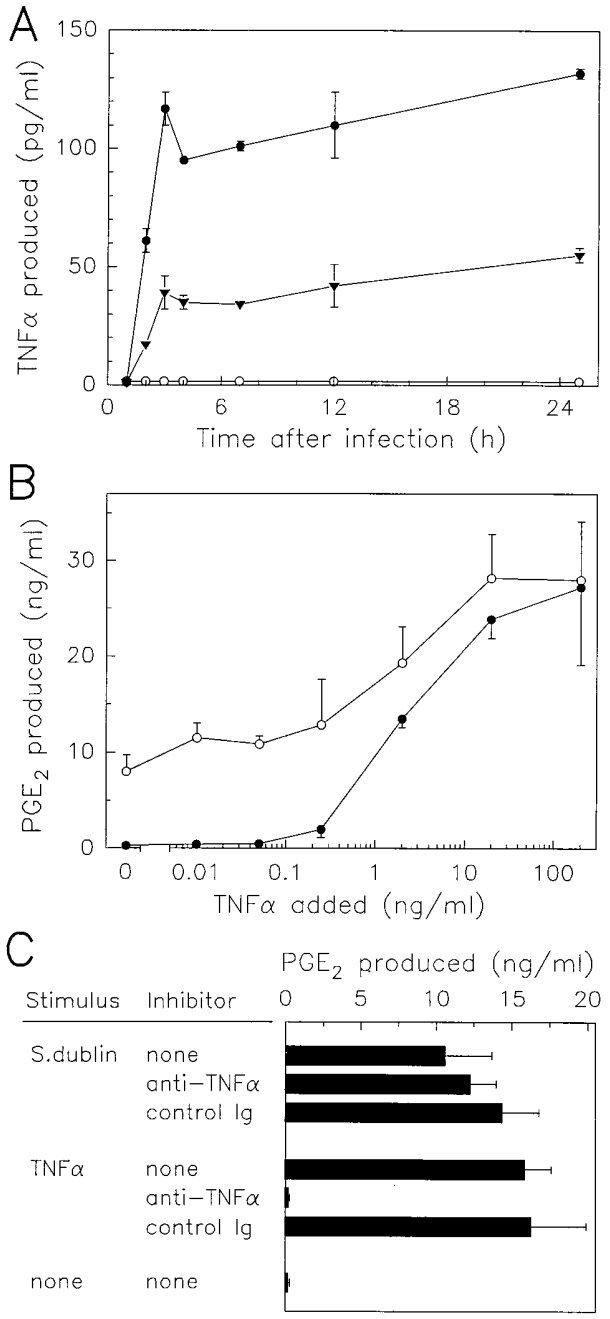

Figure 7. Role of TNFα in *Salmonella*-induced increase in PGE_2 production by HT-29 cells. (*A*) TNFα production by *Salmonella*-infected HT-29 cells. Confluent HT-29 monolayers in 6-well plates were infected for 1 h with 10^8 (●), or 1.5×10^6 (▼) *S. dublin*/well, washed, and further incubated in 1 ml/well growth medium with gentamicin. Uninfected cultures were used as controls (○). At the indicated times after infection, supernatants were removed, and TNFα levels were determined by ELISA (Quantikine HS; R & D Systems). Data are means±SD of the results from triplicate cultures. (*B*) Dose–response relationship for TNFα-induced PGE_2 production by HT-29 cells. Confluent HT-29 monolayers in 6-well plates were infected for 1 h with 10^8 *S. dublin*/well, washed, and further incubated for 7 h with gentamicin plus the indicated concentrations of TNFα (○). In parallel, uninfected cultures were stimulated with TNFα for 7 h (●). Subsequently, cultures were washed, incubated for 15 min in a 1-ml vol of DME containing 2 mg/ml BSA and 20 μM arachidonic acid, and PGE_2 levels were analyzed by specific enzyme immunoassay. Data are means±SD of the results from triplicate wells. (*C*) Neutralization of TNFα has no effect on *S. dublin*–induced PGE_2 production by HT-29 cells. Confluent HT-29 monolayers in 12-well plates were infected for 1 h with 2.5×10^7 *S. dublin*/well, washed, and further incubated for 20 h with gentamicin in the presence of 25 μg/ml of either goat anti-TNFα or normal goat IgG as a control antibody, or without added antibodies. Parallel uninfected cultures were incubated for 20 h with 5 ng/ml TNFα with and without added antibodies. PGE_2 production was assayed as described in *B* using 0.4 ml/well. Data represent means±SD of the results from triplicate cultures. Similar results were obtained for $PGF_{2\alpha}$ levels in the supernatants (data not shown).

expression in intestinal epithelial cells in control xenografts, and no upregulation after infection with *S. typhi* BRD691 (data not shown).

Supernatants from Salmonella-infected HT-29 cells stimulate increased chloride secretion by polarized T_{84} epithelial cell monolayers. To determine whether epithelial cell–derived prostaglandins can mediate the increase in fluid secretion seen after oral *Salmonella* infection of rabbits (15), culture supernatants were collected from *S. dublin*–infected and control HT-29 cell cultures, and were tested for their ability to induce chloride secretion by polarized T_{84} colonic epithelial cell monolayers. Increased chloride secretion is thought to mediate the increase in fluid secretion in infectious diarrhea, and the T_{84} model is a well-established system to study vectorial ion transport by the colonic epithelium (45). As shown in Fig. 9 *A*, conditioned media (CM) from *S. dublin*–infected HT-29 cells induced an increase in short-circuit current (I_{sc}) by polarized T_{84} cells, while CM from control HT-29 cells had no effect on the I_{sc}. The time course of the increase in I_{sc} after addition of CM from *S. dublin*–infected cells was similar to that after addition of optimal (10^{-6} M, Fig. 9 *A*) and suboptimal doses of PGE_2 (10^{-7}, 2×10^{-8}, and 10^{-9} M, data not shown). When indomethacin (3 μM) was added 1 h before CM were obtained from *S. dublin*–infected HT-29 cells, no increase in I_{sc} was observed after CM addition to the Ussing chambers (data not shown), indicating that the chloride-inducing activity in the CM was likely a product of the PGHS pathways.

To further characterize this activity, CM were preincubated with monoclonal anti-PGE_2 and tested in Ussing chambers. As shown in Fig. 9 *B*, anti-PGE_2 completely blocked the I_{sc} increase after addition of CM from *S. dublin*–infected HT-29 cells, while an isotype-matched control antibody had no effect. This suggested that PGE_2 constitutes the dominant chloride secretagogue in the CM, whereas $PGF_{2\alpha}$, which was produced at levels similar to PGE_2 (Fig. 2), appeared to have little or no role in this response. Consistent with this interpretation, $PGF_{2\alpha}$ stimulated chloride secretion by T_{84} cells with a 1,000-fold lower potency than PGE_2 (Fig. 9 *C*). Furthermore, even relatively high doses of $PGF_{2\alpha}$ (10^{-4} M) did not alter the I_{sc} response of T_{84} cells to optimal (10^{-6} M) or suboptimal (10^{-8} M) doses of PGE_2 (data not shown), indicating that $PGF_{2\alpha}$ did not modify the subsequent secretory response of T_{84} cells to PGE_2.

Discussion

Infection of intestinal epithelial cells with invasive bacteria rapidly upregulated PGHS-2 expression, and PGE_2 and $PGF_{2\alpha}$ production in vitro and in vivo. Moreover, PGE_2 produced by

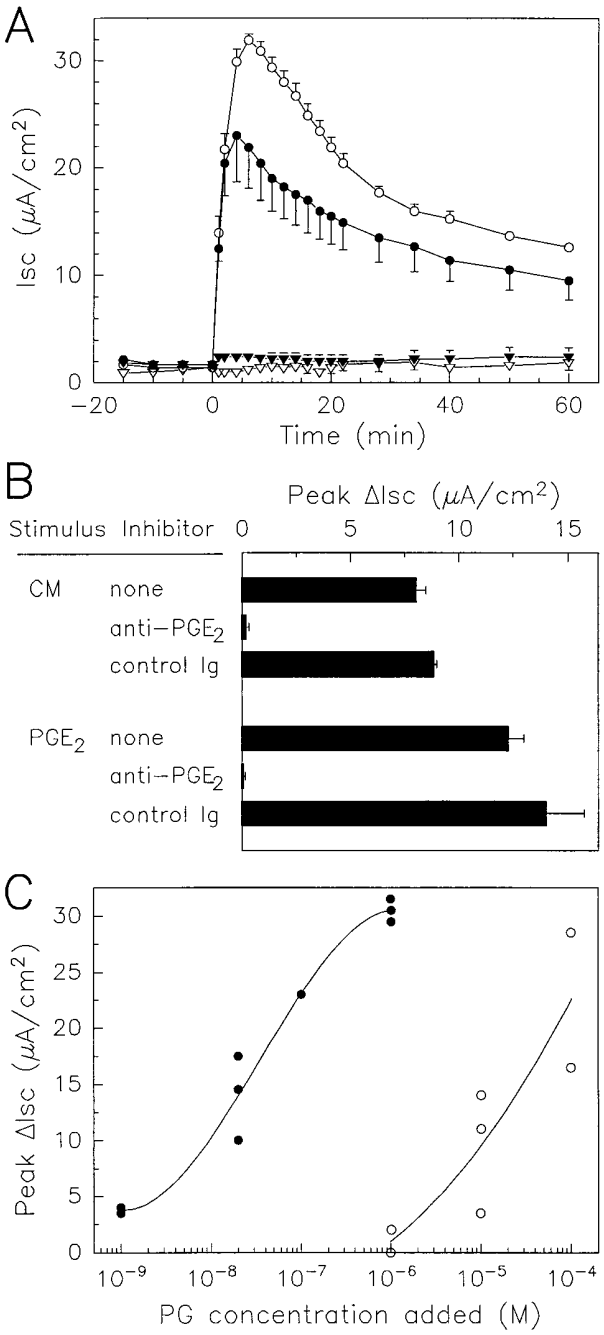

Figure 9. Supernatants from *Salmonella*-infected HT-29 cells induce chloride secretion by polarized T_{84} colon epithelial cell monolayers. (*A*) Time course of increased I_{sc} after addition of CM from *S. dublin*–infected HT-29 cells. CM from *S. dublin*–infected (●) and control HT-29 cultures (▼) were obtained as described in Methods. CM from *S. dublin*–infected cultures contained 36.5 and 57.0 ng/ml PGE_2 and $PGF_{2\alpha}$, respectively. CM from control cultures contained < 0.5 ng/ml PGE_2. Media containing 1.76 μg/ml (5×10^{-6} M) PGE_2 (○) or media alone (▽) were used as controls. Polarized T_{84} cell monolayers were mounted in Ussing chambers, equilibrated at 37°C, and background I_{sc} readings were taken for at least 20 min. Stimuli were added at 0 min on the basolateral side in a 1-ml vol to the 4-ml vol already present in the chamber (e.g., a 1:5 final dilution), and I_{sc} readings were taken initially every 2 min, later every 5 min. Data are means±SEM from the results of three independent experiments. (*B*) Anti-PGE_2 inhibits increased I_{sc} after addition of CM from *S. dublin*–infected HT-29 cells. 200 μl of CM from *S. dublin*–infected cells, or 200 μl media containing 35 ng/ml (10^{-7} M) PGE_2 was mixed with 800 μl PBS containing 75 μg monoclonal anti-PGE_2 (IgG_1 isotype), or 75 μg monoclonal mouse IgG_1 (MOPC 31c) as a control, or no added antibody. The molar ratio of antibody to PGE_2 was ~25:1. The mixtures were incubated for 20 min at room temperature, added to the basolateral side of polarized T_{84} cells mounted in Ussing chambers, and I_{sc} recordings were taken, as described in *A*. I_{sc} values from unstimulated control T_{84} monolayers were subtracted. Data are means±SEM of the peak I_{sc} values obtained in three separate experiments. (*C*) Dose–response relationships for prostaglandin-induced I_{sc} in polarized T_{84} cells. Polarized T_{84} cells were mounted in Ussing chambers, stimulated with the indicated doses of PGE_2 (●) or $PGF_{2\alpha}$ (○), and peak I_{sc} values were determined. I_{sc} values from unstimulated control T_{84} monolayers were subtracted. Each point represents a single measurement. Data from three separate experiments are shown.

bacteria-infected intestinal epithelial cells constituted the predominant activity capable of inducing chloride secretion by polarized intestinal epithelial cell monolayers. The rapid induction of epithelial PGE_2 production after bacterial entry could provide a mechanistic explanation for previous observations in animal models that infection of the intestine with invasive bacteria, including *Salmonella* and *Shigella*, rapidly induced increased fluid secretion mediated by products of the PGHS pathways (15, 21, 22). This possibility is supported by the time course of the observed host secretory response in the present study, since increased fluid secretion after *Salmonella* infection occurred within 4 h after infection in previous studies (15), a time consistent with the present finding of increased epithelial PGE_2 production within 3 h after infection. Furthermore, increased PGE_2 secretion by polarized intestinal epithelial cells was reported to occur predominantly from the basolateral surface of the cells (30), which is consistent with the previous observation that increased epithelial chloride secretion was most efficiently elicited by basolateral stimulation of polarized intestinal epithelial cells with prostaglandins (18). Although cells in the underlying mucosa, such as macrophages, can also respond to bacteria with increased PGE_2 production (46), it is unlikely that bacterial penetration of the epithelium and induction of prostaglandin production by these cells occurs sufficiently rapidly to account for the early phase of the host secretory response to bacterial infection. Nonetheless, the production of PGE_2 (and possibly other prostaglandins) by cells other than intestinal epithelial cells likely contributes to the host secretory response at later stages of the infection.

Prostaglandins can induce diarrhea in humans. Thus, administration of PGE analogues caused increased fluid secretion in volunteers (19, 47), and diarrhea caused by several noninfectious causes, including radiation therapy, was ameliorated by PGHS inhibitors (20). The role of prostaglandins in human infections with invasive bacteria, however, has not been established, although one report suggested that treatment of infants and young children with the PGHS inhibitor aspirin decreased intestinal fluid loss after infection with several enteric pathogens, including *Salmonella* (48). The apparent lack of a larger number of clinical reports linking the use of PGHS inhibitors with improvements of bacterially induced diarrhea may be related to the pleiotropic functions of prostaglandins in the intestinal tract. These include the induction of epithelial ion secretion and diarrhea (16, 19, 47) and also promotion of epithelial

wound healing (49, 50). The former would be prevented by PGHS inhibitors, while inhibition of the latter by PGHS inhibitors could lead to the exacerbation of epithelial injury, which would be likely to promote diarrhea.

Epithelial-derived prostaglandins and epithelial PGHS-2 expression may have functions during the host response to bacterial infection other than increasing fluid secretion. PGHS-2 expression is induced in other models of gastrointestinal injury, including TNBS-induced colitis in the rat (50) and gastric ulcer models in mice (51, 52), although PGHS-2 expression was not localized to epithelial cells in these studies. Products of the PGHS-2 pathway appeared to be protective in these models, since PGHS-2 inhibitors delayed wound healing in the ulcer model, and exacerbated injury in TNBS-induced colitis (50, 51). These observations raise the possibility that PGHS inhibitors might, under some circumstances, exacerbate mucosal injury after infection with invasive bacteria. It is not clear how prostaglandins exert mucosa-protective effects (49), but they are known to downregulate the production of some proinflammatory cytokines, such as IL-1, in macrophages (53). Thus, prostaglandins derived from the epithelium early in infection could function to antagonize the action of proinflammatory cytokines released from epithelial and inflammatory cells in response to infection with pathogenic bacteria. Another possible mechanism by which increased epithelial PGHS-2 expression and prostaglandin production could protect against mucosal injury is suggested by the mitogenic and antiapoptotic activity of PGHS-2 expressed in epithelial cells (30, 54). Rapid epithelial reconstitution is likely to be important for reducing mucosal access of inflammatory luminal compounds (e.g., LPS) after bacterially induced damage to the epithelium. Consistent with a mitogenic function of epithelial PGHS-2 expression, increased epithelial proliferation was seen rapidly after *Salmonella* infection of human fetal intestinal xenografts transplanted onto SCID mice (Savidge, T.C., unpublished observation). Other roles of epithelial cell-derived prostaglandins could involve the induction of mucin secretion from epithelial cells as a protective response against further bacterial invasion (55), and an increase in mucosal blood flow allowing increased numbers of inflammatory cells to reach the site of infection (56).

PGHS-2 was not constitutively expressed in intestinal epithelial cells, but increased rapidly after bacterial invasion. In contrast, PGHS-1 was constitutively expressed at low levels, and expression was not affected by bacterial infection. These data suggest that PGHS-2 is likely to be more important than PGHS-1 in mediating specific aspects of the host response to bacterial invasion. In contrast, PGHS-1 appears to be more important under normal conditions, since PGHS-1 is constitutively expressed in the normal intestinal tract, while PGHS-2 levels are low (57), but detectable with sensitive methods (58). PGHS-1 may also be more important than PGHS-2 in the host response to radiation injury (39). The respective roles of the two PGHS isoforms in the intestinal tract, however, remain to be established. Data from mice with targeted deletions of the two PGHS isoforms suggest that neither is absolutely required for normal intestinal function, since the knock-out mice had no apparent pathological changes in the gastrointestinal tract (24–26). It is unknown, however, whether these mice show abnormal gastrointestinal changes in response to mucosal injury or challenge with pathogenic enteric bacteria.

Increased epithelial PGHS-2 expression after bacterial infection initially resulted from the direct interaction of invading bacteria with the cells, and later was amplified by indirect effects, since cells not invaded by bacteria also expressed increased PGHS-2 levels, and supernatants from infected cells could stimulate increased PGE_2 production by uninfected cells. Both mechanisms probably also act in vivo, since the proportion of intestinal epithelial cells in the xenografts that expressed increased PGHS-2 levels after *Salmonella* infection is likely to be greater than the fraction of cells infected by *Salmonella.* Even after in vitro infection of intestinal epithelial cells with relatively high doses of *Salmonella*, a substantial fraction of cells remains uninfected (data not shown), and electron microscopic observations in experimentally infected intestinal loops suggested that *Salmonella* localized to a small proportion of intestinal epithelial cells (9). The identity of the PGHS-2–inducing activity is currently unknown, but based on the present studies it is unlikely to be TNFα or IL-1. Possible candidates for this activity are TGFα and amphiregulin, both of which are expressed by intestinal epithelial cells, and can induce epithelial PGE_2 production (29, 30).

In addition to PGHS-2, intestinal epithelial cells upregulate genes that encode chemokines (e.g., IL-8, MCP-1), proinflammatory cytokines (TNFα, GM-CSF), and adhesion molecules (ICAM-1) in response to infection with invasive bacteria (31–33, 38). The mechanisms underlying the coordinate expression of these genes are not known, but probably involve activation of gene transcription, since expression of reporter constructs for at least one of these genes, IL-8, was upregulated after bacterial invasion (Eckmann, L., unpublished data). Consistent with this notion, the stability of PGHS-2 mRNA was not altered after *Salmonella* infection of I407 epithelial cells (data not shown). Genes of the epithelial proinflammatory program share common features in their promoter regions, namely the presence of DNA-binding regions for the NF-κB transcription factor complex, raising the possibility that this complex is activated after infection with invasive bacteria. Consistent with this hypothesis, increased NF-κB DNA binding activity was reported in HeLa epithelial cells after infection with the invasive bacteria *S. flexneri* (59). Although such a common regulatory mechanism could account for the coordinate induction of the epithelial proinflammatory program, additional mechanisms are probably important for regulating individual genes of the program. For example, epithelial PGHS-2 induction after bacterial invasion was, in part, mediated by a released factor as shown herein, whereas such a paracrine-acting factor played little or no role in the upregulation of epithelial ICAM-1 expression after bacterial invasion (38).

The ability of the infecting bacteria to invade cells was an important determinant for the epithelial prostaglandin response, since invasive bacteria induced far greater increases in PGE_2 production than noninvasive bacteria. Infection with gram-negative, but not gram-positive, noninvasive bacteria, however, induced a small but significant increase in epithelial PGE_2 production. This increase shows that bacterial properties other than the ability to invade cells contribute to the epithelial prostaglandin response, albeit to a lesser degree than invasiveness. Data obtained in the in vivo model of normal human intestinal epithelial cells suggested that components of the gram-negative cell wall were responsible for this response, since killed (and therefore noninvasive) *S. typhi* and a live noninvasive *E. coli* were equally efficient in inducing epithelial PGHS-2 expression. Bacterial LPS may be one of the cell wall

components responsible for the increased epithelial expression of PGHS-2, as well as other proinflammatory genes (60). The intestinal epithelial cell PGE_2 response to LPS, however, was less than that to infection with gram-negative noninvasive bacteria, suggesting that cell wall components other than LPS could be important for this response. Such was the case also with gram-negative bacterial infections of bladder and lung epithelial cell cultures (61, 62). In any case, a low-level epithelial prostaglandin response to noninvasive enteric bacteria present in the normal luminal flora, such as nonpathogenic *E. coli*, suggests the concept that intestinal epithelial cells sense the presence of the normal luminal flora, and in response communicate with other epithelial cells and cells in the underlying mucosa. Such an epithelial response could contribute to the physiologic inflammation present in conventional, but not germ-free animals (63–66).

Acknowledgments

We thank Jane Smitham and Sharon Okamoto for expert technical help, and Dr. J. Portanova for providing monoclonal anti-PGE_2.

L. Eckmann is a recipient of a Career Development Award from the Crohn's and Colitis Foundation of America. D.C. Lowe is an MRC-Collaborative Student. This work was supported by National Institutes of Health grants DK35108 (M.F. Kagnoff), DK33165 (W.F. Stenson), DK28305 and DK47756 (K.E. Barrett), and the Biotechnology and Biological Sciences Research Council (T.C. Savidge).

References

1. Chalker, R.B., and M.J. Blaser. 1988. A review of human salmonellosis. III. Magnitude of *Salmonella* infection in the United States. *Rev. Infect. Dis.* 10: 111–124.

2. Centers for Disease Control. 1990. Foodborne disease outbreaks, a 5 year summary, 1983–87. *MMWR (Morb. Mortal. Wkly Rep.).* 39:15–57.

3. Stutman, H.R. 1994. *Salmonella*, *Shigella*, and *Campylobacter*: common bacterial causes of infectious diarrhea. *Pediatr. Ann.* 23:538–543.

4. Teka, T., A.S. Faruque, and G.J. Fuchs. 1996. Risk factors for deaths in under-age-five children attending a diarrhoea treatment centre. *Acta Paediatr.* 85:1070–1075.

5. DuPont, H.L. 1995. Diarrheal diseases in the developing world. *Infect. Dis. Clin. N. Am.* 9:313–324.

6. Asakura, H., and M. Yoshioka. 1994. Cholera toxin and diarrhoea. *J. Gastroenterol. Hepatol.* 9:186–193.

7. Spangler, B.D. 1992. Structure and function of cholera toxin and the related *Escherichia coli* heat-labile enterotoxins. *Microbiol. Rev.* 56:622–647.

8. Parsot, C., and P.J. Sansonetti. 1996. Invasion and the pathogenesis of *Shigella* infections. *Curr. Top. Microbiol. Immunol.* 209:25–42.

9. Jones, B.D., N. Ghori, and S. Falkow. 1994. *Salmonella typhimurium* initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. *J. Exp. Med.* 180:15–23.

10. Galan, J.E. 1996. Molecular and cellular bases of *Salmonella* entry into host cells. *Curr. Top. Microbiol. Immunol.* 209:43–60.

11. Isberg, R.R. 1996. Uptake of enteropathogenic *Yersinia* by mammalian cells. *Curr. Top. Microbiol. Immunol.* 209:1–24.

12. Galan, J.E., and R. Curtiss. 1989. Cloning and molecular characterization of genes whose products allow *Salmonella typhimurium* to penetrate tissue culture cells. *Proc. Natl. Acad. Sci. USA.* 86:6383–6387.

13. Wallis, T.S., W.G. Starkey, J. Stephen, S.J. Haddon, M.P. Osborne, and D.C. Candy. 1986. Enterotoxin production by *Salmonella typhimurium* strains of different virulence. *J. Med. Microbiol.* 21:19–23.

14. Chopra, A.K., J.W. Peterson, P. Chary, and R. Prasad. 1994. Molecular characterization of an enterotoxin from *Salmonella typhimurium. Microb. Pathog.* 16:85–98.

15. Giannella, R.A., S.B. Formal, G.J. Dammin, and H. Collins. 1973. Pathogenesis of Salmonellosis. Studies of fluid secretion, mucosal invasion, and morphologic reactions in the rabbit ileum. *J. Clin. Invest.* 52:441–453.

16. Eberhart, C.E., and R.N. DuBois. 1995. Eicosanoids and the gastrointestinal tract. *Gastroenterology.* 109:285–301.

17. Appleton, I., A. Tomlinson, and D.A. Willoughby. 1996. Induction of cyclo-oxygenase and nitric oxide synthase in inflammation. *Adv. Pharmacol.* 35: 27–78.

18. Weymer, A., P. Huott, W. Liu, J.A. McRoberts, and K. Dharmsathaphorn. 1985. Chloride secretory mechanism induced by prostaglandin E1 in a colonic epithelial cell line. *J. Clin. Invest.* 76:1828–1836.

19. Lanza, F.L., R.L. Kochman, G.S. Geis, E.M. Rack, and L.G. Deysach. 1991. A double-blind, placebo-controlled, 6-day evaluation of two doses of misoprostol in gastroduodenal mucosal protection against damage from aspirin and effects on bowel habits. *Am. J. Gastroenterol.* 86:1743–1748.

20. Mennie, A.T., and V. Dalley. 1973. Aspirin in radiation-induced diarrhea. *Lancet.* i:1131.

21. Gots, R.E., S.B. Formal, and R.A. Giannella. 1974. Indomethacin inhibition of *Salmonella typhimurium*, *Shigella flexneri*, and cholera-mediated rabbit ileal secretion. *J. Infect. Dis.* 130:280–284.

22. Giannella, R.A., W.R. Rout, and S.B. Formal. 1977. Effect of indomethacin on intestinal water transport in *Salmonella*-infected rhesus monkeys. *Infect. Immun.* 17:136–139.

23. Smith, W.L., and D.L. DeWitt. 1996. Prostaglandin endoperoxidase H synthases-1 and -2. *Adv. Immunol.* 62:167–215.

24. Morham, S.G., R. Langenbach, C.D. Loftin, H.F. Tiano, N. Vouloumanos, J.C. Jennette, J.F. Mahler, K.D. Kluckman, A. Ledford, and C.A. Lee. 1995. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. *Cell.* 83:473–482.

25. Dinchuk, J.E., B.D. Car, R.J. Focht, J.J. Johnston, B.D. Jaffee, M.B. Covington, N.R. Contel, V.M. Eng, R.J. Collins, and P.M. Czerniak. 1995. Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. *Nature (Lond.).* 378:406–409.

26. Langenbach, R., S.G. Morham, H.F. Tiano, C.D. Loftin, B.I. Ghanayem, P.C. Chulada, J.F. Mahler, C.A. Lee, E.H. Gouldin, and K.D. Kluckman. 1995. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell.* 83:483–492.

27. Harris, R.C., J.A. McKanna, Y. Akai, H.R. Jacobson, R.N. DuBois, and M.D. Breyer. 1994. Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. *J. Clin. Invest.* 94:2504–2510.

28. Holtzman, M.J. 1992. Arachidonic acid metabolism in airway epithelial cells. *Annu. Rev. Physiol.* 54:303–329.

29. DuBois, R.N., J. Awad, J. Morrow, L.J. Roberts, and P.R. Bishop. 1994. Regulation of eicosanoid production and mitogenesis in rat intestinal epithelial cells by transforming growth factor-α and phorbol ester. *J. Clin. Invest.* 93:493–498.

30. Coffey, R.J., C.J. Hawkey, L. Damstrup, R. Graves-Deal, V.C. Daniel, P.J. Dempsey, R. Chinery, S.C. Kirkland, R.N. DuBois, T.L. Jetton, and J.D. Morrow. 1997. Epidermal growth factor receptor activation induces nuclear targeting of cyclooxygenase-2, basolateral release of prostaglandins, and mitogenesis in polarizing colon cancer cells. *Proc. Natl. Acad. Sci. USA.* 94:657–662.

31. Eckmann, L., M.F. Kagnoff, and J. Fierer. 1993. Epithelial cells secrete the chemokine interleukin-8 in response to bacterial entry. *Infect. Immun.* 61: 4569–4574.

32. Jung, H.C., L. Eckmann, S.K. Yang, A. Panja, J. Fierer, E. Morzycka-Wroblewska, and M.F. Kagnoff. 1995. A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. *J. Clin. Invest.* 95:55–65.

33. McCormick, B.A., S.P. Colgan, C. Delp-Archer, S.I. Miller, and J.L. Madara. 1993. *Salmonella typhimurium* attachment to human intestinal epithelial monolayers: transcellular signalling to subepithelial neutrophils. *J. Cell Biol.* 123:895–907.

34. Barrett, K.E., and T.D. Bigby. 1993. Involvement of arachidonic acid in the chloride secretory response of intestinal epithelial cells. *Am. J. Physiol.* 264: C446–C452.

35. Chatfield, S.N., N. Fairweather, I. Charles, D. Pickard, M. Levine, D. Hone, M. Posada, R.A. Strugnell, and G. Dougan. 1992. Construction of a genetically defined *Salmonella typhi* Ty2 *aroA*, *aroC* mutant for the engineering of a candidate oral typhoid-tetanus vaccine. *Vaccine.* 10:53–60.

36. Hicks, S., D.C. Candy, and A.D. Phillips. 1996. Adhesion of enteroaggregative *Escherichia coli* to pediatric intestinal mucosa in vitro. *Infect. Immun.* 64:4751–4760.

37. Mnich, S.J., A.W. Veenhuizen, J.B. Monahan, K.C. Sheehan, K.R. Lynch, P.C. Isakson, and J.P. Portanova. 1995. Characterization of a monoclonal antibody that neutralizes the activity of prostaglandin E_2. *J. Immunol.* 155:4437–4444.

38. Huang, G.T., L. Eckmann, T.C. Savidge, and M.F. Kagnoff. 1996. Infection of human intestinal epithelial cells with invasive bacteria upregulates apical intercellular adhesion molecule-1 (ICAM-1) expression and neutrophil adhesion. *J. Clin. Invest.* 98:572–583.

39. Cohn, S.M., S. Schloemann, T. Tessner, K. Seibert, and W.F. Stenson. 1997. Crypt stem cell survival in the mouse intestinal epithelium is regulated by prostaglandins synthesized through cyclooxygenase-1. *J. Clin. Invest.* 99:1367–1379.

40. Eckmann, L., G.T. Huang, J.R. Smith, E. Morzycka-Wroblewska, and M.F. Kagnoff. 1994. Increased transcription and coordinate stabilization of mRNAs for secreted immunoglobulin α heavy chain and κ light chain following stimulation of immunoglobulin A expressing B cells. *J. Biol. Chem.* 269:33102–33108.

41. Savidge, T.C., A.L. Morey, D.J. Ferguson, K.A. Fleming, A.N. Shma-

kow, and A.D. Phillips. 1995. Human intestinal development in a severe-combined immunodeficient xenograft model. *Differentiation.* 58:361–371.

42. Futaki, N., S. Takahashi, M. Yokoyama, I. Arai, S. Higuchi, and S. Otomo. 1994. NS-398, a new anti-inflammatory, selectively inhibits prostaglandin G/H synthase/cyclooxygenase (COX-2) activity in vitro. *Prostaglandins.* 47: 55–59.

43. Bhattacharyya, D.K., M. Lecomte, J. Dunn, D.J. Morgans, and W.L. Smith. 1995. Selective inhibition of prostaglandin endoperoxidase synthase-1 (cyclooxygenase-1) by valerylsalicylic acid. *Arch. Biochem. Biophys.* 317:19–24.

44. Eckmann, L., S.L. Reed, J.R. Smith, and M.F. Kagnoff. 1995. *Entamoeba histolytica* trophozoites induce an inflammatory cytokine response by cultured human cells through the paracrine action of cytolytically released interleukin-1α. *J. Clin. Invest.* 96:1269–1279.

45. McRoberts, J.A., and K.E. Barrett. 1989. Hormone-regulated ion transport in T84 colonic cells. *In* Functional Epithelial Cells In Culture. K.S. Matlin and J.D. Valentich, editors. Alan R. Liss, Inc., New York. 235–265.

46. Yusof, W.N.W., M. Nagaratnam, C.L. Koh, S. Puthucheary, and T. Pang. 1993. Release of prostaglandin E_2 by human mononuclear cells exposed to heat-killed *Salmonella typhi. Microbiol. Immunol.* 37:667–670.

47. Matuchansky, C., J.Y. Mary, and J.J. Bernier. 1976. Further studies on prostaglandin E_1-induced jejunal secretion of water and electrolytes in man, with special reference to the influence of ethacrynic acid, furosemide, and aspirin. *Gastroenterology.* 71:274–281.

48. Gracey, M., M.A. Phadke, V. Burke, S.K. Raut, and B. Singh. 1984. Aspirin in acute gastroenteritis: a clinical and microbiological study. *J. Pediatr. Gastroenterol. Nutr.* 3:692–695.

49. Wallace, J.L., and A.W. Tigley. 1995. Review article: new insights into prostaglandins and mucosal defence. *Aliment. Pharmacol. Ther.* 9:227–235.

50. Reuter, B.K., S. Asfaha, A. Buret, K.A. Sharkey, and J.L. Wallace. 1996. Exacerbation of inflammation-associated colonic injury in rat through inhibition of cyclooxygenase-2. *J. Clin. Invest.* 98:2076–2085.

51. Mizuno, H., C. Sakamoto, K. Matsuda, K. Wada, T. Uchida, H. Noguchi, T. Akamatsu, and M. Kasuga. 1997. Induction of cyclooxygenase 2 in gastric mucosal lesions and its inhibition by the specific antagonist delays healing in mice. *Gastroenterology.* 112:387–397.

52. Stenson, W.F. 1997. Cyclooxygenase 2 and wound healing in the stomach. *Gastroenterology.* 112:645–648.

53. Knudsen, P.J., C.A. Dinarello, and T.B. Strom. 1986. Prostaglandins posttranscriptionally inhibit monocyte expression of interleukin 1 activity by increasing intracellular cyclic adenosine monophosphate. *J. Immunol.* 137:3189–3194.

54. Tsujii, M., and R.N. DuBois. 1995. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxidase synthase 2. *Cell.* 83:493–501.

55. McCool, D.J., M.A. Marcon, J.F. Forstner, and G.G. Forstner. 1990. The T84 human colonic adenocarcinoma cell line produces mucin in culture and releases it in response to various secretagogues. *Biochem. J.* 267:491–500.

56. Brown, J.A., and R.D. Zipser. 1987. Prostaglandin regulation of colonic blood flow in rabbit colitis. *Gastroenterology.* 92:54–59.

57. Kargman, S., S. Charleson, M. Cartwright, J. Frank, D. Riendau, J. Mancini, J. Evans, and G. O'Neill. 1996. Characterization of prostaglandin G/H/ synthase 1 and 2 in rat, dog, monkey, and human gastrointestinal tracts. *Gastroenterology.* 111:445–454.

58. O'Neill, G.P., and A.W. Ford-Hutchinson. 1993. Expression of mRNA for cyclooxygenase-1 and cyclooxygenase-2 in human tissues. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 330:156–160.

59. Dyer, R.B, C.R. Collaco, D.W. Niesel, and N.K. Herzog. 1993. *Shigella flexneri* invasion of HeLa cells induces NF-κB DNA-binding activity. *Infect. Immun.* 61:4427–4433.

60. Eckmann, L., H.C. Jung, C. Schurer-Maly, A. Panja, E. Morzycka-Wroblewska, and M.F. Kagnoff. 1993. Differential cytokine expression by human intestinal epithelial cell lines: regulated expression of interleukin 8. *Gastroenterology.* 105:1689–1697.

61. Hedges, S., M. Svensson, and C. Svanborg. 1992. Interleukin-6 response of epithelial cell lines to bacterial stimulation in vitro. *Infect. Immun.* 60:1295–1301.

62. DiMango, E., H.J. Zar, R. Bryan, and A. Prince. 1995. Diverse *Pseudomonas aeruginosa* gene products stimulate respiratory epithelial cells to produce interleukin-8. *J. Clin. Invest.* 96:2204–2210.

63. Van Oudenaren, A., J.J. Haaijman, and R. Benner. 1984. Frequencies of background cytoplasmic Ig-containing cells in various lymphoid organs of athymic and euthymic mice as a function of age and immune status. *Immunology.* 51:735–742.

64. Klaasen, H.L., P.J. Van der Heijden, W. Stok, F.G. Poelma, J.P. Koopman, M.E. Van den Brink, M.H. Bakker, W.M. Eling, and A.C. Beynen. 1993. Apathogenic, intestinal, segmented, filamentous bacteria stimulate the mucosal immune system of mice. *Infect. Immun.* 61:303–306.

65. Meslin, J.C., J.M. Wal, and V. Rochet. 1990. Histamine and mast cell distribution in the intestinal wall of the germ free and conventional rats. Influence of the mode of sterilization of the diet. *Agents Actions.* 29:131–137.

66. Umesaki, Y., H. Setoyama, S. Matsumoto, and Y. Okada. 1993. Expansion of alpha beta T-cell receptor-bearing intestinal intraepithelial lymphocytes after microbial colonization in germ-free mice and its independence from thymus. *Immunology.* 79:32–37.