

# Membrane peroxidation by lipopolysaccharide and iron-ascorbate adversely affects Caco-2 cell function: beneficial role of butyric acid<sup>1-3</sup>

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## ABSTRACT

**Background:** Membrane lipid peroxidation may play a role in immune-mediated bowel diseases.

**Objective:** We examined the effects of lipopolysaccharide (LPS), a ubiquitous endotoxin mediator of gram-negative bacteria, alone and in combination with iron-ascorbate, on enterocyte function. Furthermore, we assessed the antioxidant capacity of butylated hydroxytoluene (BHT) and butyric acid, which are known to play a significant role in the welfare of intestinal mucosa.

**Design:** Differentiated intestinal Caco-2 cells were used to study the induction of membrane peroxidation by LPS (100 µg/mL) and iron-ascorbate (0.2 and 2 mmol/L, respectively) and to examine the beneficial effects of BHT and butyric acid.

**Results:** A significant dose-dependent increase in malondialdehyde, accompanied by lower apical membrane fluidity and significantly decreased sucrase activity, was observed when Caco-2 cells were incubated with LPS. LPS also augmented paracellular permeability (<sup>14</sup>C]polyethylene glycol flux), prostaglandin E<sub>2</sub> production, and cyclooxygenase-2 (EC 1.14.99.1) expression. These abnormalities were exacerbated by the coadministration of iron-ascorbate, but most of them were suppressed by butyric acid and BHT.

**Conclusion:** Bacterial endotoxin and prooxidants may overwhelm antioxidant defenses and become deleterious to enterocyte function, whereas butyric acid and BHT may provide antioxidant protection. *Am J Clin Nutr* 2003;77:744-50.

**KEY WORDS** Iron-ascorbate, membrane fluidity, permeability, cyclooxygenase-2, prostaglandin E<sub>2</sub>, PGE<sub>2</sub>, lipopolysaccharide, butylated hydroxytoluene, BHT, butyric acid, Caco-2 cells

## INTRODUCTION

In addition to providing nutrient transport, the single layer of epithelial cells lining the mucosal surface imparts a functional barrier that protects against many deleterious microbial and chemical agents in the gut lumen (1). Enterocytes are also active participants in the mucosal immune response (2-7). Disruption of the host's mucosal defense system may contribute to immune-mediated bowel disorders, such as chronic inflammatory bowel diseases (IBD) and multiple organ failure (8, 9). Tissue peroxidation is a major component of the aberrant immune response in IBD (8, 10-12). Oxygen radicals elicit membrane destabilization, alterations in DNA, inactivation of proteolytic enzymes associated with the detoxification of reactive oxygen metabolites, and induction of oxidative stress via interaction with plasma and cell

factors (11). The resulting chronic intestinal inflammation promotes an imbalance between oxidant and antioxidant mechanisms at the tissue level (12), compromising circulating antioxidant reserves (13).

Excessive intestinal permeability and enteric bacterial flora have been implicated in the pathogenesis of IBD (8, 14). Lipopolysaccharide (LPS), a ubiquitous endotoxin mediator of gram-negative bacteria, can facilitate microbial translocation by a mechanism involving physical perturbation of the gut mucosal barrier (15, 16). The first aim of the present study was therefore to examine the effect of the potential interaction between free radical-induced lipid peroxidation and LPS on the integrity of the gastrointestinal mucosa. We determined the effect of LPS on intestinal epithelial permeability, transepithelial electrical resistance, fluidity, lipid peroxidation, and sucrase activity. The gastrointestinal mucosa is constantly exposed to luminal oxidants from ingested foods, desquamated cells, and bacteria (17-19). Consequently, our second aim was to evaluate the combined effects of LPS and an additional source of lipid peroxidation on the aforementioned membrane variables by using iron-ascorbate (20, 21). Short-chain fatty acids derived from dairy products and the anaerobic fermentation of carbohydrates by endogenous bacterial flora are key elements in the maintenance of human mucosal health and have potential clinical benefits in the treatment of IBD (22). Butyric acid, a short-chain fatty acid that is thought to play a significant role in the maintenance of mucosal health (23), was

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tested in terms of its capacity to scavenge reactive oxygen species generated by LPS or iron-ascorbate.

## MATERIALS AND METHODS

### Cell culture

Caco-2 cells (American Type Culture Collection, Rockville, MD) were grown at 37 °C in MEM (Gibco-BRL, Grand Island, NY) containing 1% (by vol) penicillin:streptomycin and 1% (by vol) MEM nonessential amino acids (Gibco-BRL) and supplemented with 10% (by vol) decomplexed fetal bovine serum (Flow, McLean, VA). Cells (passages 30–40) were maintained with 5% (by vol) CO<sub>2</sub> in 75-cm<sup>2</sup> flasks (T-75; Corning Inc, Acton, MA). When the cultures reached 70–90% confluence, each culture was split into 6 flasks containing 0.05% (by vol) trypsin and 0.5 mmol EDTA/L (Gibco-BRL). For individual experiments, cells were plated at a density of  $1 \times 10^6$  cells/well on 24.5mm polycarbonate Transwell filter inserts with 0.4- $\mu$ m pores (Costar, Cambridge, MA) in MEM (as above) supplemented with 5% (by vol) fetal bovine serum. The inserts were placed into 6-well culture plates, permitting separate access to the upper and lower compartments of the monolayers. Cultures were maintained for 20 d, a period in which we have observed that cells are differentiated and suitable for studies on enterocyte function, including lipid synthesis and lipoprotein secretion (24, 25). The medium was refreshed every second day.

### Estimation of lipid peroxidation

Caco-2 cells were cultured in the presence or absence of LPS and Fe<sup>2+</sup>-ascorbate, which were added to the medium in the apical compartment. The amount of free malondialdehyde (MDA) formed during the reaction was determined by HPLC as described previously (26). Proteins were first precipitated with a 10% (by vol) sodium tungstate solution (Aldrich Chemical Co, Milwaukee). The protein-free supernatant fluids were then reacted with an equivalent volume of 0.5% (wt:vol) thiobarbituric acid solution (TBA; Sigma, St Louis) at 90 °C for 30 min. After cooling to room temperature, the pink chromogene [(TBA)<sub>2</sub>-MDA] was extracted with 1-butanol and dried over a stream of nitrogen at 37 °C. The dry extract was then resuspended in a potassium dihydrogen phosphate:methanol mobile phase (70 parts:30 parts, pH 7.0) before MDA detection by HPLC.

### Effect of antioxidants

To determine whether lipid peroxidation was responsible for the alterations caused by LPS or iron-ascorbate, butylated hydroxytoluene (BHT) was added to the apical compartment for 1 h before incubation with these factors. BHT was dissolved in alcohol (1% in the medium). Control wells received an equal volume of the alcohol carrier. The efficiency of butyric acid to prevent or reduce lipid peroxidation induced by iron-ascorbate or LPS was also tested after preincubation for 20 h.

### Membrane integrity and viability of Caco-2 cells

The transepithelial membrane resistance of Caco-2 cells was assessed after exposure to LPS or Fe<sup>2+</sup>-ascorbate with a Millicell-ERS apparatus (Millipore Corp, Bedford, MA). Apical membrane sucrase activity was also measured as described previously (25, 27). [<sup>14</sup>C]Polyethylene glycol flux was evaluated after the

addition of the labeled probe (1  $\mu$ Ci/well) to the apical compartment (28). Samples were then mixed with Ready Safe Counting fluid (Beckman, Fullerton, CA), and radioactivity was quantified by scintillation counting (Beckman LS 5000 TD; Beckman, Mississauga, Ontario, Canada). Cellular protein content was determined according to the method of Lowry et al (29). Cell viability was assessed by trypan blue exclusion (30).

### Isolation of brush border membranes

Brush border membranes were purified from Caco-2 cell homogenates according to the method of Schmitz et al (31). Briefly, the culture medium was removed, and the cells attached to filters were rinsed twice with phosphate-buffered saline (Gibco-BRL). The cells were then scraped off and homogenized in tris-HCl mannitol [2 mmol HCl/L, 50 mmol mannitol/L (pH 7.0)]. After the addition of a 10-mmol MgCl<sub>2</sub>/L solution, the homogenates were centrifuged for 15 min at  $7700 \times g$  and 4 °C. The supernatant fluids were subsequently centrifuged for 30 min at  $20\,000 \times g$  and 4 °C, and the resulting pellets were used for the determination of fluidity.

### Cell membrane fluidity

Aliquots of brush border membranes were diluted with a solution containing 300 mmol mannitol/L, 10 mmol trisHEPES/L, and 100 mmol KCl/L (pH 7.5). Fluidity was estimated by incorporating the fluorescent probe 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene *p*-toluene-sulfonate and subsequently measuring polarization (26). The final molar probe-to-lipid ratio was 1:1000. Fluorescence was measured in a spectrofluorometer at 25 °C with polarization filters parallel and perpendicular to the excitation beam. Excitation was measured at 360 nm, and emission was measured at 420 nm.

### Prostaglandin E<sub>2</sub> determination

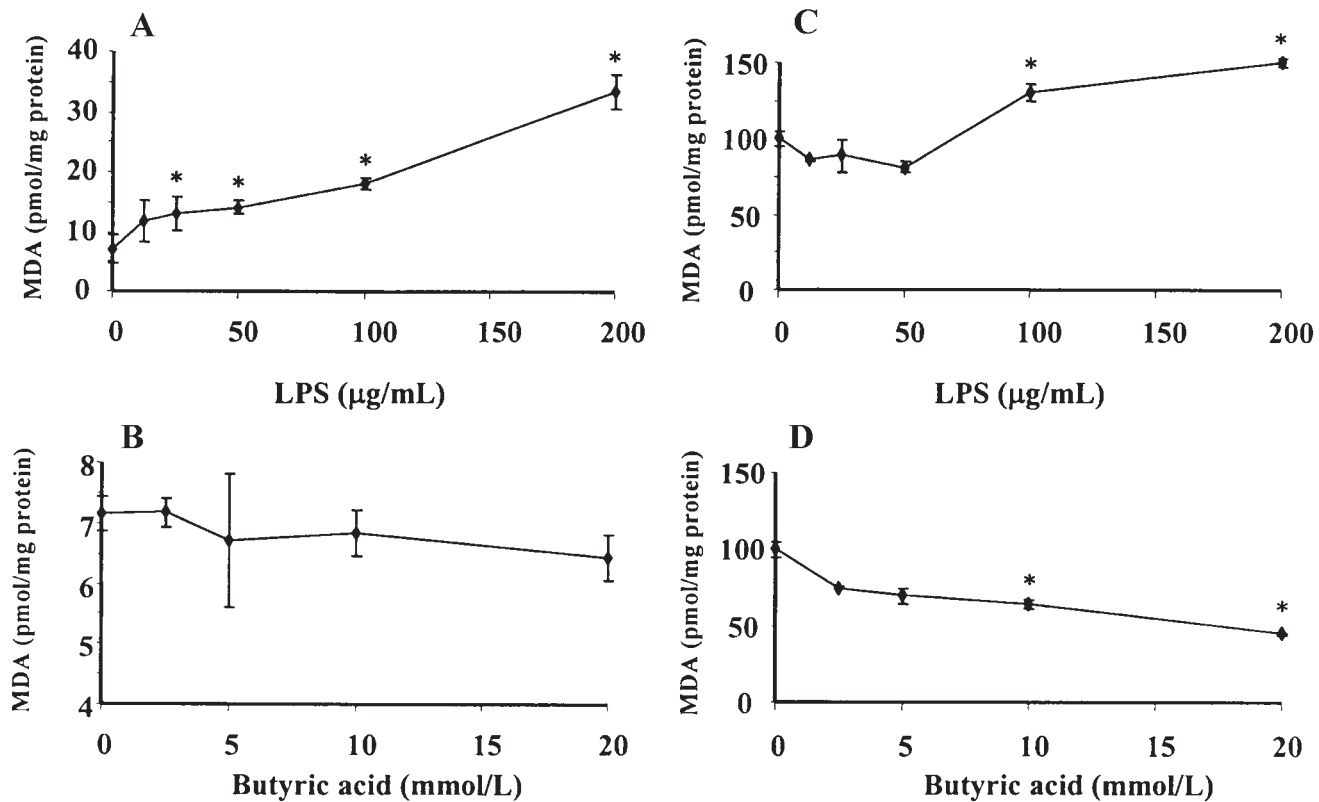
Caco-2 cells were preincubated with sodium butyrate or BHT and exposed to Fe<sup>2+</sup>-ascorbate or LPS. Cellular prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) was measured by enzyme-linked immunosorbent assay (Neogen Corp, Lansing, MI).

### Immunoblot analysis of cyclooxygenase-2

After the incubation with the antioxidants and prooxidants, Caco-2 cells were washed twice with phosphate-buffered saline and scraped in 0.5 mL lysis buffer [50 mmol tris-HCl/L (pH 7.5), 150 mmol NaCl/L, 5 mmol EDTA/L, 0.1% (by vol) sodium dodecyl sulfate, 0.5% (by vol) sodium desoxycholate, 1% (by vol) Triton (Sigma, Oakville, Ontario, Canada), 1 mmol phenylmethylsulfonyl fluoride/L, 1 mmol BHT/L, and 1 mmol pepstatin/L] as described previously (32). Cells were sonicated and aliquots of cell homogenates were mixed with loading buffer (Bio-Rad Corp, Hercules, CA). Cyclooxygenase-2 (COX-2; EC 1.14.99.1) values were determined by sodium dodecyl sulfate-polyacrylamide and transfer onto Hybond nitrocellulose membranes (Amersham, Baie d'Urfé, Québec, Canada), followed by detection with an enhanced chemiluminescence system for antigen-antibody complexes (33). Quantification was carried out by densitometry (Scion Image; Scion Corp, Frederick, MD).

### Statistical analysis

All values are expressed as means  $\pm$  SEMs. Data were analyzed by using a one-way analysis of variance and the two-tailed Student's



**FIGURE 1.** Mean ( $\pm$ SEM) malondialdehyde (MDA) concentrations (for 3 different experiments, each of which was carried out in triplicate) in Caco-2 cells (A and B) and in the apical membrane compartment (C and D) after incubation of differentiated Caco-2 cells with increasing concentrations of lipopolysaccharide (LPS) or butyric acid for 24 h at 37°C. \*Significantly different from control wells in which LPS or butyric acid was not added,  $P < 0.05$ .

*t* test. The Tukey test was used for comparisons among several groups. A  $P$  value  $< 0.05$  was considered significant. Results were analyzed by using BMDP NEW SYSTEM for WINDOWS (version 1.0; BMDP Statistical Software, Inc, Los Angeles).

## RESULTS

### Membrane lipid peroxidation

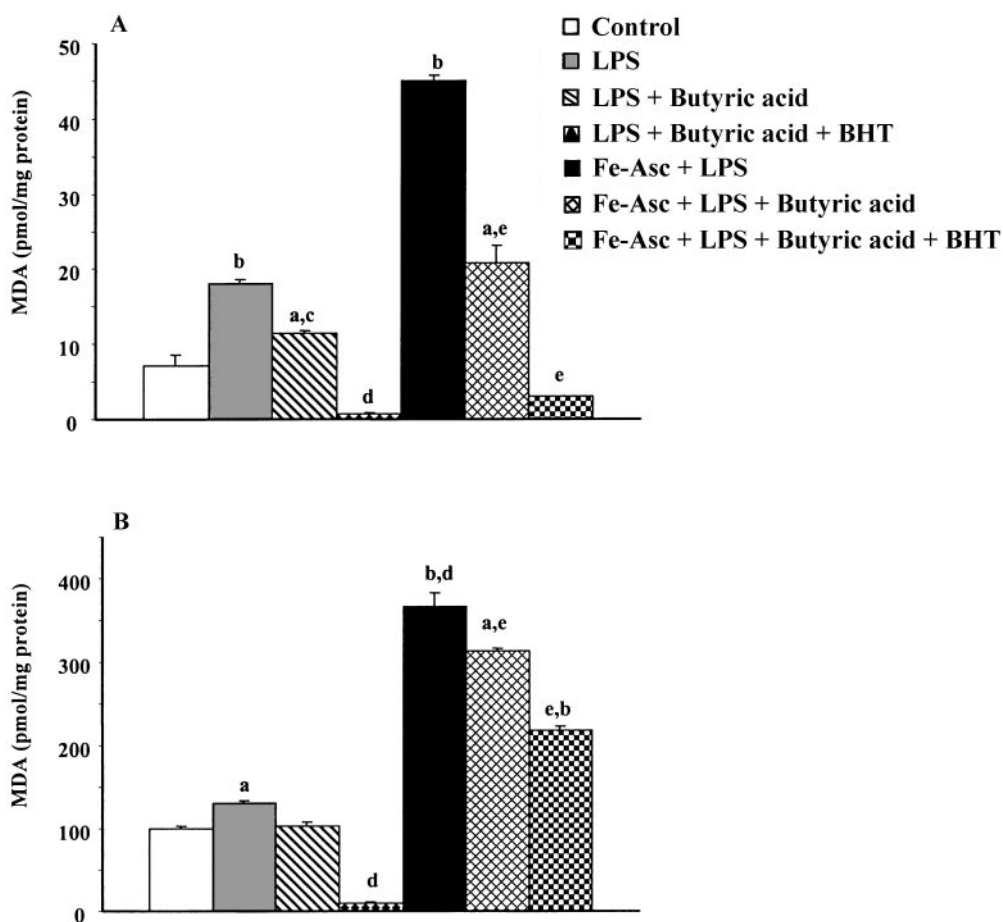
LPS facilitates the binding of bacteria to cell surfaces and the delivery of bacterial toxins. Short-chain fatty acids are major byproducts of anaerobic bacterial metabolism that are able to diffuse across biological membranes (22). Thus, differentiated Caco-2 cells were used to study the induction of membrane peroxidation by LPS and the potential protective role of butyric acid. As shown in **Figure 1**, when Caco-2 cells were incubated with various concentrations of LPS for 24 h, the MDA content in the apical compartment and in the cells was significantly higher than when cells were not incubated with LPS. The exposure of Caco-2 cells to butyric acid did not significantly alter MDA production. The combination of LPS (100  $\mu$ g/mL) and iron-ascorbate (0.2 and 2 mmol/L, respectively), an established oxygen free radical-generating system, significantly augmented MDA concentrations (**Figure 2**). The addition of butyric acid significantly decreased LPS and iron-ascorbate-mediated lipid peroxidation, which was totally suppressed in Caco-2 cells and significantly reduced in the apical compartment by the administration of BHT (200  $\mu$ mol/L).

### Functional integrity and viability of Caco-2 cells

The effect of LPS on Caco-2 membrane integrity and cell viability was assessed by monolayer transepithelial resistance, sucrase activity, protein content, and trypan blue exclusion after an incubation period of 24 h (data not shown). Transepithelial resistance and protein content remained unchanged with increasing concentrations of LPS. Similarly, cell viability was unaffected by the addition of LPS (data not shown). Only sucrase activity moderately, but significantly, decreased in response to LPS, at concentrations between 25 and 200  $\mu$ g/mL. Butyric acid did not result in significant alterations in any of these variables (data not shown). Further experiments showed that the combination of LPS and iron-ascorbate did not amplify the derangements observed with the administration of LPS alone. However, butyric acid neutralized the decrease in sucrase activity elicited by LPS or LPS with iron-ascorbate (data not shown).

### Membrane fluidity

Experiments were carried out to determine the effects of LPS, iron-ascorbate, and butyric acid on membrane fluidity, indicative of the relative motional freedom of lipid molecules in the membrane bilayer. The degree of fluidity of surface membrane lipids can be estimated by fluorescence polarization analyses with the probe 1,6-diphenyl-1,3,5-hexatriene. Fluidity was thus determined as the reciprocal of 1,6-diphenyl-1,3,5-



**FIGURE 2.** Mean ( $\pm$ SEM) malondialdehyde (MDA) concentrations (for 3 different experiments, each of which was carried out in triplicate) in Caco-2 cells (A) and in the apical membrane compartment (B) after differentiated Caco-2 cells were preincubated with butyric acid, butylated hydroxytoluene (BHT), or both for 24 h before being incubated with lipopolysaccharide (LPS; 100  $\mu$ g/mL), iron-ascorbate (Fe-Asc; 0.2 and 2 mmol/L, respectively), or both for an additional 24 h. <sup>a,b</sup>Significantly different from control wells: <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ . <sup>c,d</sup>Significantly different from LPS: <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.001$ . <sup>e</sup>Significantly different from LPS+Fe-Asc,  $P < 0.05$ .

hexatriene fluorescence polarization in apical membranes isolated from Caco-2 cells exposed to LPS, iron-ascorbate, or butyric acid (data not shown). A trend toward a reduction in membrane fluidity was noted with LPS alone. Iron-ascorbate alone, or combined with LPS, significantly decreased membrane fluidity. Butyric acid and BHT restored membrane fluidity values to those of the control cells in the presence of LPS and iron-ascorbate.

#### Paracellular membrane permeability

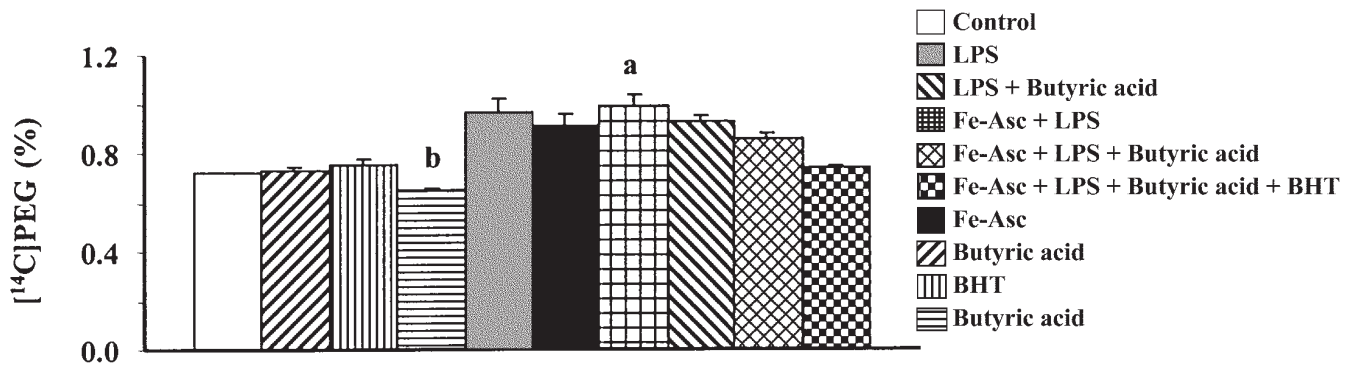
Polyethylene glycol uptake is considered to be a sensitive tool to estimate the paracellular permeability of the intestinal epithelium. To determine whether LPS or iron-ascorbate affects tight junctions, [<sup>14</sup>C]polyethylene glycol was added to the apical compartment of Caco-2 cell cultures. Caco-2 cells incubated with LPS or iron-ascorbate alone showed a trend toward higher [<sup>14</sup>C]polyethylene glycol uptake than that in cells not incubated with LPS or iron-ascorbate (Figure 3). The combination of these 2 factors resulted in a significant increase in permeation flux. This alteration decreased with the administration of butyric acid, BHT, or the mixture of butyric acid and BHT.

#### Prostaglandin E<sub>2</sub> production and cyclooxygenase protein expression

COX-2 is a highly regulated enzyme that catalyzes the production of prostaglandins under physiologic and pathologic conditions (34). As shown in Figure 4, Caco-2 cells that were incubated with LPS or iron-ascorbate had significantly higher PGE<sub>2</sub> concentrations than did those that were not incubated with LPS or iron-ascorbate. The most significant change was obtained with the combination of LPS and iron-ascorbate. PGE<sub>2</sub> concentrations returned to normal with the coadministration of butyric acid and BHT. COX-2 protein concentrations were examined by Western blot (Figure 5). Caco-2 cells that were incubated with LPS, iron-ascorbate, or the 2 prooxidants combined had higher COX-2 values than did those that were not incubated. Neither butyric acid nor BHT alone decreased COX-2 protein values after incubation of Caco-2 cells with LPS and iron-ascorbate. However, decreased COX-2 values were noted with the coadministration of butyric acid and BHT.

#### DISCUSSION

Intestinal epithelial cells are a key component to the physiologic barrier between the myriad of toxins, microbes, and antigens



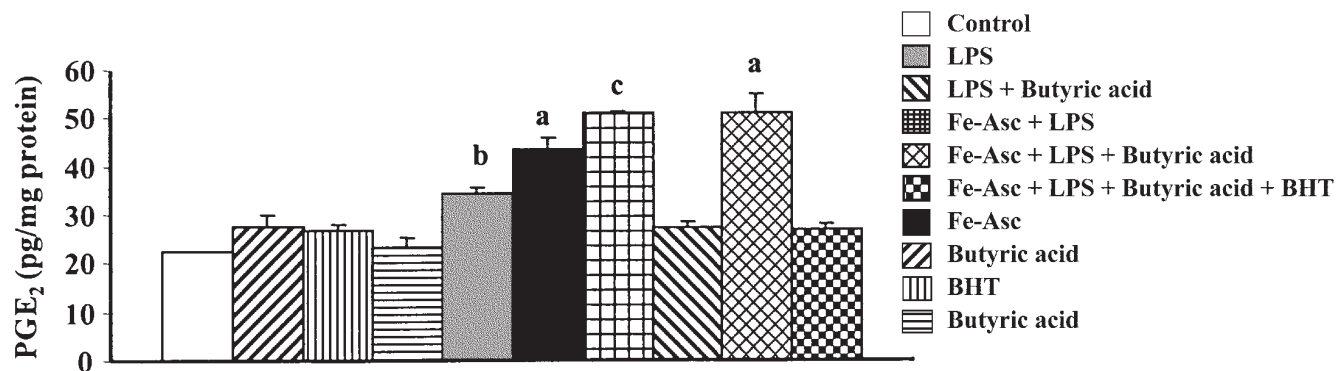
**FIGURE 3.** Mean ( $\pm$ SEM) [ $^{14}$ C]polyethylene glycol (PEG) permeation (for 3 different experiments) in differentiated Caco-2 cells that were or were not pretreated with butyric acid (2.5 mmol/L), butylated hydroxytoluene (BHT; 200  $\mu$ mol/L), or both for 24 h before incubation with [ $^{14}$ C]PEG in the presence or absence of lipopolysaccharide (LPS; 100  $\mu$ g/mL), iron-ascorbate (Fe-Asc; 0.2 and 2 mmol/L, respectively), or both for an additional 24 h at 37  $^{\circ}$ C. <sup>a,b</sup>Significantly different from control wells: <sup>a</sup> $P < 0.04$ , <sup>b</sup> $P < 0.01$ .

present in the gut lumen and the underlying mucosal immune system (1, 2). The activated immune cells residing in the lamina propria and interspersed between epithelial cells have traditionally been considered to be the key effector cellular elements in the host's mucosal immune response. However, there is increasing evidence that enterocytes are actively involved as full participants in generating an immune response at the mucosal level and are not just the targets of inflammation (35). The major purpose of the present set of experiments was to ascertain how microbial and peroxidative factors alter intestinal epithelial cell barrier function. Exposure of Caco-2 cells to LPS or Fe<sup>2+</sup>-ascorbate resulted in marked changes in MDA concentrations, membrane lipid fluidity, sucrase activity, and paracellular permeability. Furthermore, these agents induced changes in PGE<sub>2</sub> concentrations and COX-2 protein expression. Butyric acid and BHT restored mucosal barrier function and the activity and protein expression of COX-2 to normal values.

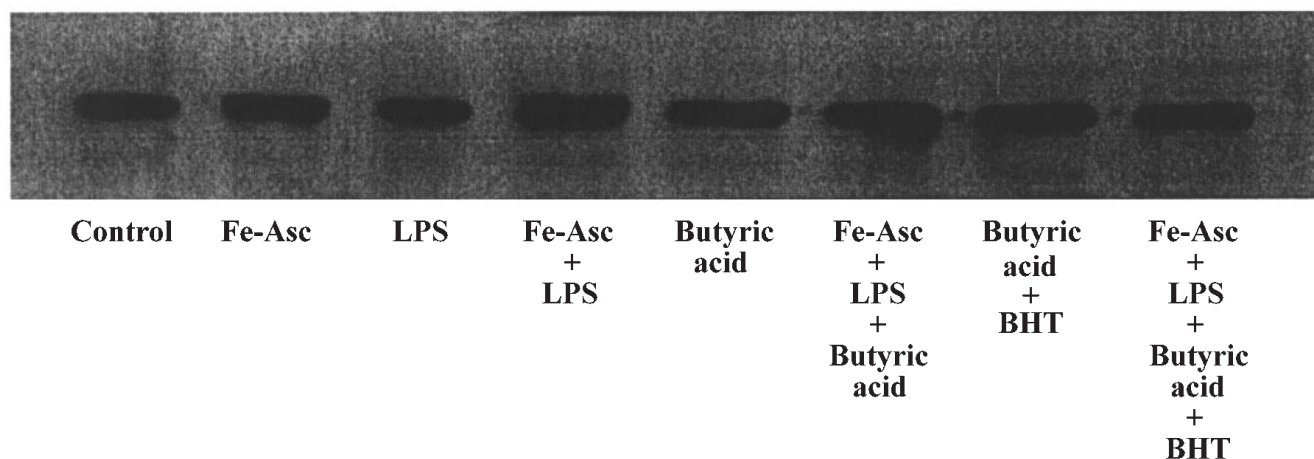
Gram-negative bacterial species constitute the main source of endotoxin in the gut microflora (34). LPS is a potent stimulus of the host's immune response via its capacity to induce the proinflammatory cytokine cascade. LPS characteristically activates neutrophils, monocytes, and macrophages via its CD14

membrane receptor (34). An increasing body of evidence points to the direct effect of LPS on intestinal epithelial cells via toll-like receptors (36). LPS can induce cell proliferation (37), modulate energy metabolism (38), and augment the production of inflammatory cytokines by enterocytes (39). The results outlined in the present report provide evidence that LPS is also able to induce enterocyte lipid peroxidation and concomitantly alter the membrane lipid fluidity, paracellular permeability, and sucrase activity of enterocytes.

Zareie et al (40) described an in vitro model of inflammation in which coculture of confluent monolayers of human T84 intestinal epithelial cells with LPS-activated monocytes resulted in significant abnormalities in epithelial ion transport and barrier function. Because the antioxidant BHT prevented these effects in our experiments, it is reasonable to suggest that impairment of membrane integrity is caused by LPS-mediated lipid peroxidation. A potential mechanism may involve peroxidative attack on polyunsaturated fatty acids in the membrane, altering the membrane phospholipid environment, which, in turn, may influence membrane function. No matter what the initiating event in inflammatory bowel diseases may be, the driving oxidative force provided by luminal microorganisms



**FIGURE 4.** Mean ( $\pm$ SEM) prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) concentrations measured by enzyme-linked immunosorbent assay (for 3 different experiments) in differentiated Caco-2 cells that were or were not pretreated with butyric acid (2.5 mmol/L), butylated hydroxytoluene (BHT; 200  $\mu$ mol/L), or both for 24 h before incubation with lipopolysaccharide (LPS; 100  $\mu$ g/mL), iron-ascorbate (Fe-Asc; 0.2 and 2 mmol/L, respectively), or both for an additional 24 h at 37  $^{\circ}$ C. <sup>a-c</sup>Significantly different from control wells: <sup>a</sup> $P < 0.04$ , <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.005$ .




**FIGURE 5.** Cyclooxygenase-2 (COX-2) expression analyzed by Western blot (of 3 culture experiments) in differentiated Caco-2 cells that were or were not pretreated with butyric acid (2.5 mmol/L), butylated hydroxytoluene (BHT; 200  $\mu$ mol/L), or both for 24 h before incubation with lipopolysaccharide (LPS; 100  $\mu$ g/mL), iron-ascorbate (Fe-Asc; 0.2 and 2 mmol/L, respectively), or both for an additional 24 h at 37 °C. Mean ( $\pm$ SD) scanning values (in arbitrary units) were as follows: control, 7438  $\pm$  668; Fe-Asc, 8310  $\pm$  845; LPS, 9692  $\pm$  600; Fe-Asc+LPS, 12 405  $\pm$  256; butyric acid, 7616  $\pm$  128; Fe-Asc+LPS+butyric acid, 11 686  $\pm$  234; butyric acid+BHT, 7910  $\pm$  44; and Fe-Asc+LPS+butyric acid+BHT, 7979  $\pm$  44.

may damage the intestinal mucosa by means of the microorganisms' products, such as LPS. The resulting breach of epithelial barrier function would facilitate the translocation of bacterial antigens and the production of proinflammatory mediators by the host's mucosal cells.

An important issue is whether the luminal endotoxins released from bacteria belonging to the normal gut flora or from enteropathogenic microorganisms interact with ingested prooxidants to amplify epithelial barrier perturbations. An extensive body of literature documents the ability of combined iron and ascorbate molecules to initiate substantial lipid peroxidation in native and reconstituted biological membrane systems. Our previous studies (20, 21) showed the oxidative potential of iron-ascorbate to modify the membrane compartment of enterocytes and hepatocytes, thereby impairing their lipid transport and metabolism. In the present set of experiments, Fe<sup>2+</sup>-ascorbate effectively induced Caco-2 cell lipid peroxidation, as evidenced by high concentrations of MDA, a commonly used indicator of lipid peroxidation. Moreover, the prooxidant combination of LPS and Fe<sup>2+</sup>-ascorbate caused synergistic derangements to membrane integrity. Our data suggest that further clinical trials should be undertaken to interfere with the production of peroxidative stress in patients with IBD (41).

The therapeutic role of short-chain fatty acids in various intestinal disorders is still under evaluation. Their use is well established in diversion colitis, providing evidence that the shortage of intraluminal short-chain fatty acids is associated with long-standing inflammation (42, 43). In keeping with this, our findings indicate that butyric acid may help overcome oxidative stress induced by LPS, thus preventing gut injury. Additional studies are needed to elucidate the mechanisms involved in butyric acid-induced repair processes, in view of its multiple effects on the intestinal mucosa. In addition to being an important nutrient, butyric acid also acts as a cofactor for the regulation of transcription proteins involved in gene expression and interacts with G-proteins at the membrane-cytoplasm interface (44). Furthermore, butyric acid has a marked trophic effect on normal colonic mucosa, stimulates epithelial proliferation through neural and hormonal mechanisms, and aug-

ments mucosal weight, DNA content, and crypt length in normal bowel. The opposite effects are induced in colonic neoplastic cell lines, with arrest of the cells in the G1 phase and inhibition of DNA synthesis.

Cyclooxygenase exists as 2 isoenzymes, COX-1 and COX-2. Compelling evidence suggests that COX-1 synthesizes prostaglandins involved in the regulation of normal cell activity, whereas COX-2 seems to primarily produce prostaglandins at sites of inflammation (45). Studies showed that oxidative status correlates with increases in the COX-derived prostanoid biosynthetic process (46). In the present study, we found that LPS enhanced COX-2 activity and protein values, which were accompanied by higher MDA concentrations and abnormalities in membrane integrity. Interestingly, butyric acid and BHT reduced lipid peroxidation and simultaneously suppressed COX-2 activity and protein values. Taken together, our data suggest that the prooxidant and antioxidant effects of LPS and butyric acid, respectively, may directly influence COX-2. 

## REFERENCES

1. DeWitt RC, Kudsk KA. The gut's role in metabolism, mucosal barrier function, and gut immunology. *Infect Dis Clin North Am* 1999; 13:465-81.
2. Dionne S, Ruemmele FR, Seidman EG. Immunopathogenesis of inflammatory bowel disease: role of cytokines and immune cell-enterocyte interactions. In: Bistrrian BR, Walker-Smith JA, eds. *Inflammatory bowel diseases*. Nestlé nutrition workshop series. Clinical & performance programme, vol 2. Basel, Switzerland: Karger, 1999:41-57.
3. Jobin C, Hellerbrand C, Licato LL, Brenner DA, Sartor RB. Mediation by NF-kappa B of cytokine induced expression of intercellular adhesion molecule 1 (ICAM-1) in an intestinal epithelial cell line, a process blocked by proteasome inhibitors. *Gut* 1998;42: 779-87.
4. Li XC, Jevnikar AM, Grant DR. Expression of functional ICAM-1 and VCAM-1 adhesion molecules by an immortalized epithelial cell clone derived from the small intestine. *Cell Immunol* 1997;175: 58-66.
5. Ruemmele FM, Dionne S, Levy E, Seidman EG. TNFalpha-induced

- IEC-6 cell apoptosis requires activation of ICE caspases whereas complete inhibition of the caspase cascade leads to necrotic cell death. *Biochem Biophys Res Commun* 1999;260:159–66.
6. Ruemmele FM, Dionne S, Levy E, Seidman EG. Dexamethasone inhibits IFN $\gamma$ -induced MHC class II expression of intestinal epithelial cells independently of the TGF- $\beta$ 1 regulatory pathway. *Aliment Pharmacol Ther* 1999;13:595–601.
  7. Waterhouse CC, Stadnyk AW. Rapid expression of IL-1 $\beta$  by intestinal epithelial cells in vitro. *Cell Immunol* 1999;193:1–8.
  8. Papadakis KA, Targen SR. Current theories on the causes of inflammatory bowel disease. *Gastroenterol Clin North Am* 1999;28:283–96.
  9. Swank GM, Deitch EA. Role of the gut in multiple organ failure: bacterial translocation and permeability changes. *World J Surg* 1996;20:411–7.
  10. Ma TY. Intestinal epithelial barrier dysfunction in Crohn's disease. *Proc Soc Exp Biol Med* 1997;214:318–27.
  11. Verspaget HW, Mulder TPJ, van der Sluys Veer A, Pena AS, Lamers CBHW. Reactive oxygen metabolites and colitis: a disturbed balance between damage and protection. *Scand J Gastroenterol Suppl* 1991;188:44–51.
  12. Buffinton GD, Doe WF. Depleted mucosal antioxidant defences in inflammatory bowel disease. *Free Radic Biol Med* 1995;19:911–8.
  13. Levy E, Rizwan Y, Thibault L, et al. Altered lipid profile, lipoprotein composition and oxidant/antioxidant status in pediatric Crohn's disease. *Am J Clin Nutr* 2000;71:807–15.
  14. Sartor RB. Role of the enteric microflora in the pathogenesis of intestinal inflammation and arthritis. *Aliment Pharmacol Ther* 1997;11:17–22.
  15. Wells CL, Jechorek RP, Olmsted SB, Erlandsen SL. Effect of LPS on epithelial integrity and bacterial uptake in the polarized human enterocyte-like cell line Caco-2. *Circ Shock* 1993;40:276–88.
  16. Parks DA. Oxygen radicals: mediators of gastrointestinal pathophysiology. *Gut* 1989;30:293–8.
  17. Nourooz-Zadeh J, Appelquist LA. Cholesterol oxides in Swedish foods and food ingredients: milk powder products. *J Food Sci* 1988;53:74–9.
  18. Wahle KW, Hoffe PP, McIntosh G. Effects of storage and various intrinsic vitamin E concentrations on lipid oxidation in dried egg powders. *J Sci Food Agric* 1993;61:463–9.
  19. Parks DA, Williams TK, Beckman JS. Conversion of xanthine dehydrogenase to oxidase in ischemic rat intestine: a re-evaluation. *Am J Physiol* 1988;254:G768–74.
  20. Courtois F, Suc I, Garofalo C, Ledoux M, Seidman E, Levy E. Iron/ascorbate-mediated lipid peroxidation alters the efficiency of caco-2 cells to assemble and secrete lipoproteins. *Am J Physiol* 2000;279:G12–9.
  21. Brunet S, Thibault L, Lepage G, Seidman EG, Dubé N, Levy E. Modulation of endoplasmic reticulum-bound cholesterol regulatory enzymes by iron/ascorbate-mediated lipid peroxidation. *Free Radic Biol Med* 2000;28:46–54.
  22. Mortensen PB, Clausen MR. Short-chain fatty acids in the human colon: relation to gastrointestinal health and disease. *Scand J Gastroenterol* 1996;31:132–48.
  23. Scheppach W, Sommer H, Kirchner T, et al. Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. *Gastroenterology* 1992;103:51–6.
  24. Spalinger JH, Seidman EG, Lepage G, Ménard D, Gavino V, Levy E. Uptake and metabolism of a structured triglyceride by Caco-2 cells: reversal of essential fatty acid deficiency. *Am J Physiol* 1998;275:G652–9.
  25. Mehran M, Levy E, Bendayan M, Seidman E. Lipid, apolipoprotein, and lipoprotein synthesis and secretion during cellular differentiation in Caco-2 cells. *In Vitro Cell Dev Biol Anim* 1997;33:118–28.
  26. Brunet S, Thibault L, Delvin E, Yotov W, Bendayan M, Levy E. Dietary iron overload and induced lipid peroxidation are associated with impaired plasma lipid transport and hepatic sterol metabolism in rats. *Hepatology* 1999;29:1809–17.
  27. Mehran M, Seidman E, Marchand R, Gurbindo C, Levy E. Tumor necrosis factor- $\alpha$  inhibits lipid and lipoprotein transport by Caco-2 cells. *Am J Physiol* 1995;269:G953–60.
  28. Rao RK, Baker RD, Baker SS, Gupta A, Holycross M. Oxidant-induced disruption of intestinal epithelial barrier function: role of protein tyrosine phosphorylation. *Am J Physiol* 1997;273:G812–23.
  29. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265–75.
  30. Baker SS, Baker RD Jr. Caco-2 cell metabolism of oxygen-derived radicals. *Dig Dis Sci* 1993;38:2273–80.
  31. Schmitz J, Preiser H, Mastracci D, Ghosh BK, Cerda JJ, Crane RK. Purification of the human intestinal brush border membrane. *Biochim Biophys Acta* 1973;323:98–112.
  32. Tsujii M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc Natl Acad Sci U S A* 1997;94:3336–40.
  33. Stan S, Delvin E, Rouleau T, Steinmetz A, Levy E. Modulation of apo A-IV transcript levels and synthesis by n-3, n-6 and n-9 fatty acids in Caco-2 cells. *J Cell Biochem* 1999;75:73–81.
  34. Ulevitch RJ, Tobias PS. Recognition of gram-negative bacteria and endotoxin by the innate immune system. *Curr Opin Immunol* 1999;11:19–22.
  35. Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology* 1998;115:182–205.
  36. Cario E, Rosenberg IM, Brandwein SL, Beck PL, Reinecker HC, Podolsky DK. Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing toll-like receptors. *J Immunol* 2000;164:966–72.
  37. Chakravorty D, Kumar KS. Modulation of barrier function of small intestinal epithelial cells by lamina propria fibroblasts in response to lipopolysaccharide: possible role of TNF alpha in inducing barrier dysfunction. *Microbiol Immunol* 1999;43:527–33.
  38. Fujushima K, Sasaki I, Takahashi K, Naito H, Matsuno S. Lipopolysaccharide- and proinflammatory cytokine-induced energy production in intestinal and colonic epithelial cell lines. *Scand J Gastroenterol* 1999;34:291–6.
  39. Ogle CK, Guo X, Hasselgren PO, Ogle JD, Alexander JW. The gut as a source of inflammatory cytokines after stimulation with endotoxin. *Eur J Surg* 1997;163:45–51.
  40. Zareie M, McKay DM, Kovarik GG, Perdue MH. Monocyte/macrophages evoke epithelial dysfunction: indirect role of tumor necrosis factor- $\alpha$ . *Am J Physiol* 1998;275:C932–9.
  41. Seidman EG, Bernotti S, Levy E. Nutritional modulation of gut inflammation. In: Labadarios D, Pichard C, eds. *Clinical nutrition: early intervention*. Nestlé nutrition workshop series. Clinical & performance programme, vol 7. Basel, Switzerland: Karger, 2002.
  42. Scheppach W, German-Australian SCFA study group. Treatment of distal ulcerative colitis with short-chain fatty acid enemas. *Dig Dis Sci* 1996;41:2254–9.
  43. Tonelli F, Dolara P, Batignani G, et al. Effects of short chain fatty acids on mucosal proliferation and inflammation of ileal pouches in patients with ulcerative colitis and familial polyposis. *Dis Colon Rectum* 1995;38:974–8.
  44. Velquez OC, Ederer HM, Rombeau JL. Butyrate and the colonocyte. Implications for neoplasia. *Dig Dis Sci* 1996;41:727–39.
  45. Crofford LJ, Lipsky PE, Brooks P, Abramson SB, Simmon LS, van de Putte LB. Basic biology and clinical application of specific cyclooxygenase-2 inhibitors. *Arthritis Rheum* 2000;43:4–13.
  46. Kim H, Kim K, Yu B, Chung H. The effect of age on cyclooxygenase-2 gene expression. NF- $\kappa$ B activates IkappaB $\alpha$  degradation. *Free Radic Biol Med* 2000;28:683–92.