Modulation of cellular phospholipid fatty acids and leukotriene B₄ synthesis in the human intestinal cell (CaCo-2)

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Abstract

The ability of a human colonocyte epithelial cell line (CaCo-2) to synthesise leukotriene B₄ was examined. In addition, the effects of stimulation with calcium ionophore, inhibition by a drug which specifically prevents activation of 5-lipoxygenase, and modification of the fatty acid composition of membrane phospholipids on leukotriene B₄ synthesis were assessed. Incubation with calcium ionophore (A23187) resulted in a dose and time dependent increase in leukotriene B₄ synthesis. After cell phospholipids had been enriched with oleic acid, linoleic acid, and arachidonic acid, leukotriene B₄ synthesis was found to be increased 3.2-fold, 5.5-fold, and 6.1-fold above control. Treatment with MK-886 inhibited leukotriene B₄ synthesis by 79% to 94% in the various groups. Variations in the polyunsaturated fatty acid content of intestinal epithelial cells could be important in the modulation of cellular responses. We have shown for the first time in this human intestinal epithelial cell its ability to synthesise leukotriene B₄. In addition, leukotriene B₄ synthesis can be modulated by the fatty acid composition of membrane phospholipids, which can be altered by dietary fatty acids. The synthesis of chemotactic factors, such as leukotriene B₄, by the mucosal epithelium may contribute to the recruitment of granulocytes into the colonic mucosa and across the epithelium, giving rise to the crypt abscesses which characterise ulcerative colitis.

Leukotrienes are potent inflammatory mediators derived from arachidonic acid primarily through the actions of the enzymes phospholipase A₂ and 5-lipoxygenase.¹ In recent years, considerable evidence has been accumulated to support a role for leukotrienes, particularly leukotriene B₄, in the pathogenesis of inflammatory bowel disease.² Leukotriene B₄ has potent chemotactic, chemokinetic and proaggregatory effects on neutrophils, and has been shown to account for the majority of chemotactic activity which can be extracted from biopsies of inflamed mucosa from ulcerative colitis patients.³ Furthermore, the synthesis of leukotriene B₄ has been shown to be markedly raised in the colon of patients with inflammatory bowel disease.²

The cellular sources of leukotriene B₄ synthesis in the colon have not been identified.⁴ There is evidence that the infiltrating granulocyte is the major source of leukotriene B₄ synthesis in the inflamed colon of the rat.⁵ Depletion of circulating neutrophils with an anti neutrophil serum, however, did not significantly affect resting levels of colonic leukotriene B₄ synthesis,⁶

suggesting that leukotriene B₄ is also produced by other cells in the colon. One possible source is the colonic epithelium, which has been shown to synthesise prostaglandins and thromboxanes.⁷ Isolated rat colonocytes have been shown to produce leukotriene B₄ as well as other eicosanoids.⁸

In the present study, we have used the CaCo-2 cell line as a model of human enterocytes in order to address the possibility that the epithelium is an important source of leukotriene B₄ synthesis. CaCo-2 cells are an enterocytic cell line derived from a human colonic adenocarcinoma, and have been widely used as a model of the human intestinal epithelium.^{9,10} In culture, these cells spontaneously differentiate to resemble enterocytes with functional and morphological characteristics of enteric villous type epithelia.¹¹⁻¹³ In addition to examining the possibility that CaCo-2 cells can synthesise leukotriene B₄, we have tested the effects of stimulation with calcium ionophore, inhibition by a drug which prevents activation of 5-lipoxygenase, and modification of the fatty acid composition of membrane phospholipids.

Methods

CELL CULTURE

The human colonic adenocarcinoma cell line – CaCo-2 was obtained from American Type Culture Collection (Rockville, MD) and was cultured in 25 mm² flasks (Falcon) or six well plates (Nunc). All tissue culture materials were obtained from Gibco Canada (Toronto, Ont). The cells were grown in RPMI-1640, supplemented with 2 mM glutamine, 1 mM pyruvate, 2% sodium bicarbonate, penicillin (100 IU/ml), streptomycin (100 µg/ml), gentamicin sulphate (50 µg/ml), amphotericin B (2.5 µg/ml), and 15% fetal calf serum (Gibco, Canada). Cultures were maintained at 37°C in a 5% CO₂, 95% O₂ atmosphere. They were subcultured every 10 days after exposure to 0.25% trypsin-EDTA with a plating density of 1:5 (4–5 × 10⁵ cells/ml). Cultures generally reached confluence by the sixth day after subculture. All experiments were performed on cells between passages 19 and 25. Fresh media was exchanged every two days before confluence and daily thereafter. For the described experiments, culture media (enriched with 100 µmol/l of a specific fatty acid), was replaced four days after cultures became confluent. All fatty acid enrichments were performed in this manner for 10 days. Cell viability, as assessed by trypan blue exclusion (and in some cases by electron micro-

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scopy), was unaffected by any of the experimental procedures.

100 μM FATTY ACID ALBUMIN MEDIA

PREPARATION

Sodium salts of the fatty acids were obtained from Sigma Chemicals (St Louis, Mo, USA). Stock solutions of oleic acid (18:1 n-9), linoleic acid (18:2 n-6) and arachidonic acid (20:4 n-6), in 95% ethanol (containing 0.0004% BHT), were kept under nitrogen at -20°C. The necessary aliquot of stock solution was evaporated completely under nitrogen, dissolved in 1 ml RPMI-1640 heated to 56°C and rapidly added to 10 ml of albumin-RPMI containing the appropriate amount of albumin to maintain at 3:1 fatty acid:albumin ratio (100 μmol/l:33 μmol/l), under constant stirring at 37°C. This was then filtered (0.45 μm), before being adjusted with RPMI-1640 containing 2.5% fetal calf serum (as described under 'cell culture'), so that the final concentration of fatty acid enrichment was 100 μmol/l. 'Control' media were prepared essentially as described above except no fatty acid was added. Fatty acid enriched media was freshly prepared every three days and stored at 4°C.

TABLE I Fatty acid composition of the cell culture media

Media fatty acid	Control	Oleic	Linoleic	Arachidonic
		Mole % Composition		
C14:0	2.0	0.9	1.0	0.3
C16:0	24.7	6.5	6.9	6.5
C18:0	24.5	5.4	3.8	5.8
C18:1n-9	16.6	68.0	4.4	4.7
C18:2n-6	5.6	3.5	70.1	3.5
C18:3n-3	0.8	0.9	0.3	0.6
C20:0	1.2	1.9	1.3	1.2
C20:1n-9	1.5	0.8	0.5	0.9
C20:3n-6	2.4	1.1	1.2	0.9
C20:4n-6	7.2	2.1	1.6	68.9
C20:5n-3	0.4	0.3	0.2	0.4
C22:0	0.9	1.5	1.1	0.9
C22:5n-3	1.7	0.8	0.6	1.0
C22:6n-3	1.8	1.7	1.1	1.4
Other*	8.8	4.6	5.9	3.0
Unsaturation index†	97.8	110.6	172.6	311.2

The control media contained RPMI with 2.5% fetal calf serum. The oleic acid (18:1n-9), linoleic acid (18:2n-6) and arachidonic acid (20:4n-6) media contained RPMI with 2.5% fetal calf serum supplemented with 100 μM fatty acid, respectively. Aliquots of culture media were analysed for fatty acid composition by gas chromatography. Data shown are the mean relative per cent composition of fatty acids from three different experiments. The SEM was in every case less than 10%. *Other: includes the sum of all other fatty acids (not shown) that were less than <0.5% of total fatty acids. †Unsaturation index: is the sum of the number of double bonds in each unsaturated fatty acid species.

TABLE II Major phospholipid subclass composition of CaCo-2 cell

Major phospholipid subclass	Control	Oleic	Linoleic	Arachidonic
% PC	52.6 (3.3)	53.4 (4.0)	50.8 (4.6)	45.4 (4.2)
nmol/mg	247.6 (15.7)	305.1 (12.9)	306.9 (16.6)	279.4 (20.8)
% PE	19.3 (1.1)	23.4 (1.1)*	23.2 (1.3)*	29.6 (1.7)*
nmol/mg	91.0 (5.2)	133.5 (6.2)*	139.8 (7.2)*	181.9 (10.5)*
% PS	16.6 (1.2)	11.4 (0.9)*	11.0 (1.6)*	5.7 (0.3)*
nmol/mg	78.2 (5.4)	64.9 (4.9)*	66.5 (5.4)*	35.3 (2.0)*
% PI	8.4 (0.6)	8.9 (0.7)	12.4 (1.1)*	15.6 (1.5)*
nmol/mg	39.7 (2.8)	48.6 (7.1)	74.8 (9.3)*	96.2 (16.4)*
% SM	3.0 (0.9)	3.1 (0.7)	2.6 (0.5)	3.7 (0.6)
nmol/mg	14.3 (2.8)	17.5 (3.1)	15.6 (3.0)	22.5 (4.3)

Total lipids were extracted from CaCo-2 cell homogenates, and individual phospholipid subclasses were separated as described in the methods. The phospholipid concentration was calculated for each subclass from their fatty acid concentration which was determined by gas liquid chromatography. Data are presented as mean (SEM) % phospholipid composition (top), and nmol phospholipid/mg protein (bottom) from four different determinations. PC: phosphatidylcholine; PE: phosphatidylethanolamine; PS: phosphatidylserine; PI: phosphatidylinositol; SM: spingomyelin. * Denotes means that are significantly different (p<0.05) from the control group.

PREPARATION OF MK-886, CALCIUM IONOPHORE (A23187)

MK-886 {3-[1-(4-chlorobenzyl)-3-t-butyl-thio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid} kindly supplied by Dr A W Ford-Hutchinson of Merck-Frosst, Canada (Kirkland, Quebec, Canada) and the calcium ionophore (A23187; BMC, Montreal, Canada) were dissolved in 95% ethanol at a concentration of 10 mg/ml and stored at -20°C until use. The appropriate aliquot of stock solution was evaporated completely under nitrogen, dissolved in 1 ml RPMI-1640 heated to 56°C and rapidly added to albumin-RPMI, under constant stirring as described for fatty acid albumin media.

CALCIUM IONOPHORE (A23187) STIMULATION

CaCo-2 monolayers grown on six-well plates were incubated at 37°C in a metabolic shaker with A23187 concentrations ranging from 0.5 to 10 μmol/l for 15 and 30 minutes. At the end of these times the monolayers were rinsed with ice cold 0.25% albumin phosphate buffer, and suspended in 1 ml of the same buffer. Cells were scraped off the plate, cell suspensions kept on ice and homogenates were prepared by sonication. Aliquots were then taken for leukotriene B₄ and protein analysis.

INHIBITION OF LEUKOTRIENE SYNTHESIS BY MK-886

MK-886 at a final concentration of 10 μmol/l was prepared and added to the fatty acid enriched media as described above. Media containing final concentrations of both fatty acid (100 μmol/l) and MK-886 (10 μmol/l) were added to CaCo-2 cultures for the latter half of the enrichment period (usually for five days) before stimulation with calcium ionophore (10 μmol/l for 30 minutes). 'Control' media was prepared essentially as described containing MK-886, but without fatty acid enrichment. Cells were harvested and homogenates prepared as described above. Aliquots were then taken for leukotriene B₄ and protein analysis.

LEUKOTRIENE AND PROTEIN ASSAYS

The amount of leukotriene B₄ in the cell homogenates was determined by a specific radioimmunoassay as described in detail previously.⁵ The antiserum crossreacts at <0.05% with other metabolites or arachidonic acid.

High performance liquid chromatography

To validate the efficacy of our radioimmunoassay leukotriene B₄ assay, attempts were made to characterise the leukotriene B₄ synthesised by these cells. Lipooxygenase products were extracted from cell homogenates and separated by reversed phase high performance liquid chromatography in the laboratory of Dr P Borgeat (Universal of Laval, Quebec, Canada), as previously described.¹⁴ Fractions of the mobile phase were collected at one minute elution intervals during the course of high performance liquid chromatography run (0-16 minutes). As

previously authentic leukotriene standards monitored by absorbance at 280 nm were shown to coelute within fractions four to 13 minutes, these were used to measure leukotriene B₄

immunoreactivity by our leukotriene B₄ radio-immunoassay.

Protein was assayed by the Bradford method,¹⁵ and leukotriene B₄ homogenate levels were normalised to their protein content.

TABLE III Total phospholipid fatty acid composition of CaCo-2 cells

Major fatty acid	Control	Oleic	Linoleic	Arachidonic
		Mole % Composition		
14:0	2.1 (0.1)	1.5 (0.5)	1.7 (0.1)	1.6 (0.1)
16:0	20.9 (1.3)	17.8 (1.9)	19.8 (1.0)	20.5 (1.7)
16:1n-7	7.0 (0.4)	4.7 (0.4)*	4.2 (0.3)*	3.9 (0.3)*
18:0	12.2 (1.1)	11.2 (0.5)	12.0 (0.9)	10.2 (1.9)
18:1n-9	27.9 (2.6)	39.2 (2.5)*	20.2 (1.2)	20.5 (1.0)
18:1n-7	12.8 (1.0)	9.3 (0.8)	9.1 (0.6)	9.1 (0.8)
18:2n-6	1.0 (0.1)	0.8 (0.2)	14.7 (1.3)*	4.7 (0.2)*
20:4n-6	6.7 (0.7)	5.0 (0.3)	9.1 (0.5)*	16.4 (1.2)*
22:5n-6	2.0 (0.8)	1.0 (0.3)	1.5 (0.5)	1.9 (0.6)
22:6n-3	3.2 (1.1)	2.5 (0.9)	2.0 (0.8)	2.2 (0.9)
ΣSaturates	36.1 (3.7)	31.1 (3.9)	35.2 (2.9)	35.5 (2.9)
ΣUnsaturates	63.9 (4.8)	68.9 (3.8)	64.8 (3.6)	64.5 (3.9)
Σn-7	19.8 (1.6)	14.0 (1.2)*	13.3 (0.8)*	13.0 (1.0)*
Σn-9	29.3 (2.0)	43.7 (3.0)*	20.7 (1.2)	20.8 (1.7)
Σn-6	9.1 (1.2)	7.0 (0.6)	26.8 (2.9)*	26.1 (2.5)*
Σn-3	5.7 (1.1)	4.0 (0.3)	3.9 (0.4)	4.6 (0.4)
Unsaturation index:	115.0 (7.9)	112.6 (4.4)	127.7 (8.6)*	151.8 (11.4)*

The fatty acid composition of CaCo-2 cell total phospholipids were analyzed by gas liquid chromatography. Data are presented as the relative mean (SEM) % fatty acid composition from four separate experiments * Denotes means that are significantly different (p<0.05) from the control group.

Figure 1: Leukotriene B₄ synthesis (ng/mg) by CaCo-2 cell monolayers after incubation for (○—○) 15 minutes; or (●—●) 30 minutes with concentrations of calcium ionophore A23187, ranging from 0.5 μmol/l to 10 μmol/l. Means (SEM) of three separate experiments.

Figure 2: Leukotriene B₄ synthesis (ng/mg) in CaCo-2 cell monolayers enriched with various fatty acids before (■), no inhibitor and after (▨) treatment with 10 μmol/l MK-886. Means (SEM) of six separate experiments. (*) Denotes groups that are significantly different (p<0.05) from control group.

LIPID ANALYSIS

Fatty acids

Lipids were extracted from aliquots of fatty acid enriched media or cell homogenates by the method of Folch *et al.*¹⁶ The fatty acids were methyl transesterified by the method of Lepage and Roy.¹⁷ The resulting fatty acid methyl esters were separated by gas liquid chromatography using a Supelcowax 10 capillary column (Supelco, Canada), and a temperature programmed run from 150° to 240°C. Under these conditions fatty acids identified from standard mixtures ranged from C14:0 to C22:6. C15:0 was used as the internal standard and authentic standards were run to identify all compounds quantified by gas liquid chromatography.

Phospholipids

Separation of phospholipid species was performed using one dimensional thin layer chromatography, according to the method of Skipski *et al.*¹⁸ Lipid extracts from cell homogenates were spotted onto silica gel H plates and developed using chloroform methanol acetic acid water (50:30:8:4, vol/vol). The plates were carefully dried in nitrogen flushed tanks, visualised by fluorescence, and identified by retention factors of known standards run on the same plate. Appropriate regions of silica were then scraped into reaction tubes, containing C15:0 as internal standard and the phospholipid fatty acids were methylated as above. Fatty acid methyl esters were then extracted from the silica gel and injected onto the gas liquid chromatograph. The relative fatty acid composition and mole weight per cent of each phospholipid species was estimated using the method of Christie *et al.*¹⁹

STATISTICAL ANALYSIS

All data are expressed as the mean (SEM). Comparisons between groups were calculated with the Student's *t* test for non-paired data. With all statistical analyses, an associated probability (*p* value) of ≤5% was considered significant.

Results

ENRICHMENT MEDIA FATTY ACID COMPOSITION
The fatty acid composition of the control, oleic acid enriched, linoleic acid enriched and arachidonic acid enriched media are shown in Table I. Oleic acid, linoleic acid and arachidonic acid comprised approximately 68%–70.1% of total fatty acids comparison with the control media, which contained 16.6% oleic acid, 5.6% linoleic acid, and 7.2% arachidonic acid.

Figure 3: High performance liquid chromatography (HPLC) profile of immunoreactive leukotriene B₄ standard with blank (upper panel), and CaCo-2 cell homogenate extracts before and after treatment with MK-886 (lower panel). Extracts from CaCo-2 cell homogenates demonstrated significantly large immunoreactivity in an eluate collection period cochromatographing with the leukotriene B₄ standard, and this was subsequently markedly attenuated after pretreatment of the cells with a specific 5-lipoxygenase inhibitor MK-886.

PHOSPHOLIPID AND FATTY ACID COMPOSITION AFTER ENRICHMENT

The mole quantity, and per cent fatty acid composition of major phospholipid subspecies found in CaCo-2 cell homogenates are shown in Table II. Compared with the control, enrichment of the media with oleic, linoleic, or arachidonic acids caused significant increases in the mole concentrations of phosphatidylcholine and phosphatidylethanolamine with a decrease in phosphatidylserine. The concentration of phosphatidylinositol was significantly raised from control, most notably with linoleic acid or arachidonic acid supplementation of the media. Media fatty acid supplementation also caused changes from control in the relative percentages of the phospholipid subspecies (Table II). The relative percentage of phosphatidylethanolamine increased, and phosphatidylserine decreased, with all three unsaturated fatty acid enrichments, whereas phosphatidylinositol increased only with linoleic or arachidonic acid supplementation, when compared with control.

The fatty acid mole per cent composition in CaCo-2 cell total phospholipids of CaCo-2 cell homogenates are shown in Table III. As expected, enrichment of the media with oleic acid, linoleic acid or arachidonic acid significantly increased these respective fatty acids in the total cell phospholipids compared with control. In addition linoleic acid supplementation caused an increase in the arachidonic acid, whereas arachidonic acid supplementation resulted in an increase in linoleic acid content of cell total phospholipids. In contrast with significant changes in monounsaturated and polyunsaturated fatty acids, saturated fatty acid levels were not significantly altered from control in the phospholipids of these cell homogenates (Table III).

LEUKOTRIENE B₄ SYNTHESIS IN CACO-2 HOMOGENATES AFTER STIMULATION

In three separate experiments CaCo-2 cells were stimulated by adding calcium ionophore (A23187) at concentrations ranging from 0.5 to 10 µmol/l. After 15 to 30 minutes leukotriene B₄

was measured in cell homogenates. As shown in Figure 1, there was a dose and time dependent increase in leukotriene B₄ synthesis after adding the ionophore. Because the highest rates of synthesis of leukotriene B₄ was found in cells after a 30 minutes incubation with 10 µmol/l A23187, these conditions were subsequently used to investigate leukotriene B₄ synthesis after cell membranes were enriched with various fatty acids.

EFFECTS OF DIETARY FATTY ACID ENRICHMENT AND MK-886 TREATMENT ON LEUKOTRIENE B₄ SYNTHESIS IN CACO-2 HOMOGENATES

In six separate experiments CaCo-2 cells were cultured in oleic acid, linoleic acid and arachidonic acid enriched media (Fig 2). The synthesis of leukotriene B₄ measured in these cells after stimulation with A23187, was found to be increased 3.2-fold, 5.5-fold, and 6.1-fold from control after cell phospholipids had been enriched with oleic acid, linoleic acid, and arachidonic acid, respectively. The increase leukotriene B₄ synthesis in these cells after treatment with MK-886 was inhibited 94.1%, 85.6%, 89.1% and 79.2% in control, oleic acid, linoleic acid, and arachidonic acid enriched cells, respectively.

Figure 3 shows the reversed phase high performance liquid chromatography analysis. In the top figure a standard containing 12.4 ng of leukotriene B₄ (with a corresponding blank run), gave a maximised immunoreactive peak eluting at fraction number 8. In cell homogenates (bottom figure) run under identical conditions, we were able to resolve a coeluting immunoreactive leukotriene B₄ fraction. In addition in cells that were pretreated with a specific 5-lipoxygenase inhibitor MK-886, the leukotriene B₄ immunoreactivity coeluting in this fraction was markedly attenuated. These experiments thus established the presence of immunoreactive leukotriene B₄ synthesised by these cells as resolved and separated by an alternate method high performance liquid chromatography.

Attempts were also made to measure leukotriene B₄ concentrations in the medium. After the 30 minute stimulation period with A23187, we were unable to detect significantly increased levels of leukotriene B₄ in the medium. Leukotriene B₄ levels were measurable, however, in the cell media of unstimulated cells. For example, control (71 (12) pg/ml), oleic (128 (18) pg/ml), linoleic (147 (35) pg/ml) and arachidonic (295 (32) pg/ml).

Discussion

In this study we have shown for the first time that a human colonocytic epithelial cell line (CaCo-2) is capable of synthesising leukotriene B₄. In addition, the synthesis of leukotriene B₄ after stimulation with calcium ionophore can be modulated by altering the fatty acid phospholipid composition, or by inhibiting intracellular 5-lipoxygenase activity. MK-886 has a mechanism of action distinct from previously described 5-lipoxygenase inhibitors.²⁰ It appears to specifically block activation of 5-lipoxygenase by

inhibiting the translocation of this enzyme from the cytosol to the plasma membrane.²¹

Leukotriene B₄ is a potent proinflammatory, chemokinetic, and chemotactic agent that is believed to modulate inflammatory responses in the gastrointestinal tract.²³ While CaCo-2 cells have recently been shown to actively metabolise lipoxygenase products, though β -oxidation,²⁰ there have been no previous reports that these cells can synthesise eicosanoids. It has been demonstrated that intestinal biopsies of patients with inflammatory bowel disease are capable of synthesising large amounts of prostaglandin E₂, thromboxanes, and leukotrienes.⁷ Also it has been reported that isolated rat colonocytes are capable of synthesising leukotriene B₄, in addition to other eicosanoids.⁸ Whereas the primary cellular source of leukotriene B₄ in experimental colitis is the neutrophil,⁶ there is scant information on sources of LTB₄ production in the normal mucosa.

The results of our study are consistent with previous reports showing that the CaCo-2 cell is capable of incorporating into its cellular phospholipids significant levels of exogenously added fatty acids.^{23,24} Our data show that membrane phospholipid molar concentrations as well as fatty acid composition are substantially modified by growing these cells in fatty acid enriched media. The results of the present study are generally consistent with data previously obtained in other cell lines, reviewed by Spector and Yorek.²⁵ Specifically n-6 (18:2n-6 or 20:4n-6) polyunsaturated fatty acid enrichment increases the molar concentrations of phosphatidylethanolamine and phosphatidylinositol, relative to phosphatidylserine, while simultaneously enriching the fatty acid composition of these phospholipids. Similarly, normal tissues of animals and humans can also be altered by changes in dietary fats.²⁶

Modification of the availability of fatty acid substrate by nutritional means clearly provides a mechanism for altering leukotriene B₄ synthesis. The arachidonic acid content of cell phospholipids were increased by supplementation of arachidonic acid to the culture media. Feeding intestinal cells with linoleic acid (18:2n-6), an essential fatty acid also showed increased leukotriene B₄ synthesis, of a magnitude similar to feeding arachidonic acid (20:4n-6). The increased availability of arachidonic acid would suggest the presence and activity of intestinal fatty acid desaturases and elongases in these epithelial cells because arachidonate is the product, as well as the precursor substrate for the synthesis of the series-4-leukotrienes. The demonstration of leukotriene B₄ synthesis in response to calcium ionophore stimulation, coupled with a direct inhibition of this synthesis by a specific 5-lipoxygenase inhibitor effectively suggests that these human intestinal cells of epithelial origin are capable of participation in the inflammatory response. The fact that we were unable to detect significantly high levels of leukotriene B₄ in the media just after stimulation may be because of the highly polarised nature of these CaCo-2 cell monolayers,¹⁰⁻¹² since we did not attempt stimulation experiments on monolayers grown on filters.

We have shown that an epithelial cell line in culture exhibits remarkable similarities to other inflammatory cells. These cells readily incorporate free fatty acids into membrane phospholipids,²⁷ and can modify exogenous fatty acids by elongation and desaturation.²⁸ Furthermore agonist stimulated deacylation of cellular phospholipids and the endogenous release of free arachidonate results in the synthesis of leukotriene B₄, which may be attenuated by selective inhibition of 5-lipoxygenase activity.

If these results can be extrapolated to human intestinal epithelial cells, there may be important implications in terms of our understanding of intestinal inflammation. The epithelium may be a source of potent chemotactic factors, such as leukotriene B₄, and may therefore actively participate in the recruitment of granulocytes. Production of such factors by the epithelium would also contribute to the migration of granulocytes across the epithelial layer, giving rise to the crypt abscesses which characterise ulcerative colitis. Finally, if the colonic epithelium is an important source of leukotriene B₄ production in inflammatory bowel disease, an explanation may be at hand for the apparently divergent observations that intraluminal 5-aminosalicylic acid decreases the level of leukotriene B₄ in the colon,²⁹ yet 5-ASA does not gain access to the mucosa in significant concentrations to account for such an effect.³⁰

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- Laursen LS, Naesdal J, Bukhave K, Lauritsen K, Rask-Madsen J. Selective 5-lipoxygenase inhibition in ulcerative colitis. *Lancet* 1990; 335: 683-5.
- Stenson WF. Role of eicosanoids as mediators in inflammatory bowel disease. *Scand J Gastroenterol* 1990; 25: (suppl 172): 13-8.
- Lobos EA, Sharon P, Stenson WF. Chemotactic activity in inflammatory bowel disease. Role of leukotriene B₄. *Dig Dis Sci* 1987; 32: 1380-8.
- Dreyling KW, Hoppe U, Peskar BA, Morgenroth K, Kozuschek W, Peskar BM. Leukotriene synthesis by human gastrointestinal tissues. *Biochim Biophys Acta* 1986; 878: 184-93.
- Wallace JL, Keenan CM. An orally active inhibitor of leukotriene synthesis accelerates healing in a rat model of colitis. *Am J Physiol* 1990; 258: G527-34.
- Wallace JL, Keenan CM. Leukotriene B₄ potentiates colonic ulceration in the rat. *Dig Dis Sci* 1990; 35: 622-9.
- Zifroni A, Treves AJ, Sachar DB, Rachmilewitz D. Prostanoid synthesis by cultured epithelial and mononuclear cells in inflammatory bowel disease. *Gut* 1983; 24: 659-64.
- Craven PA, DeRubertis FR. Profiles of eicosanoid production by superficial and proliferative colonic epithelial cells and sub-epithelial colonic tissue. *Prostaglandins* 1986; 32: 387-99.
- Hildago IJ, Raub TJ, Borchart RT. Characterization of the human colon carcinoma cell line (CaCo-2) as a model system for intestinal epithelial cell permeability. *Gastroenterology* 1989; 96: 736-49.
- Chantret I, Barbat A, Dussaulx E, Brattain MG, Zweibaum A. Epithelial polarity, villin expression, and enterocyte differentiation of cultured human colon carcinoma cells: a survey of twenty cell lines. *Cancer Res* 1988; 48: 1936-42.
- Pinto M, Robine-Leon S, Appay M-D, Kedinger N, Triadou E, Dussaulx E, et al. Enterocyte-like differentiation and polarization of the human colon carcinoma cell line CaCo-2 in culture. *Biol Cell* 1983; 47: 323-30.
- Van't Hof W, Van Meer G. Generation of lipid polarity in intestinal epithelial (CaCo-2) cells: Spingolipid synthesis in the Golgi complex and sorting before vesicular traffic to the plasma membrane. *J Cell Biol* 1990; 111: 977-86.
- Hughes TE, Sasak WV, Ordoval JM, Forte TM, Lamou-Fava S, Schaefer EJ. A novel cell line (CaCo-2) for the study of intestinal lipoprotein synthesis. *J Biol Chem* 1987; 262: 3762-7.
- Borgeat P, Picard S, Vallerand P, Bourgois S, Odeimat A, Sirois P, et al. Automated on-line extraction and profiling of lipoxygenase products of arachidonic acid by high-performance liquid chromatography. *Methods Enzymol* 1990; 187: 98-116.

- 15 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248-54.
- 16 Folch JM, Lees M, Sloane GH. A simple method for isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957; 226: 497-509.
- 17 Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. *J Lipid Res* 1986; 27: 114-20.
- 18 Skipski VP, Peterson RF, Barclay M. Separation of phosphatidylethanolamine, phosphatidylserine and other phospholipids by thin-layer chromatography. *J Lipid Res* 1962; 3: 467-70.
- 19 Christie WW, Noble RC, Moore JH. Determination of lipid classes by a gas-chromatographic procedure. *Analyst* 1970; 95: 940-4.
- 20 Gillard J, Ford-Hutchinson AW, Chan C, Charleson S, Denis A, Foster A, *et al.* L-663,53 6(MK-886)(3-[1-(4-chlorobenzyl)-3-t-butyl-thiol-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid), a novel, orally active leukotriene biosynthesis inhibitor. *Can J Physiol Pharmacol* 1989; 67: 456-64.
- 21 Rouser CA, Ford-Hutchinson AW, Morton HE, Gillard JW. MK-886, a potent and specific leukotriene biosynthesis inhibitor blocks and reverses the membrane association of 5-lipoxygenase in ionophore-challenged leukocytes. *J Biol Chem* 1990; 265: 1436-42.
- 22 Riehl TE, Bass NM, Stenson WF. Metabolism of 15-hydroxyeicosatetraenoic acid by CaCo-2 cells. *J Lipid Res* 1990; 31: 773-80.
- 23 Dahsti N, Smith EA, Alaupovic P. Increased production of apolipoprotein B and its lipoproteins by oleic acid in CaCo-2 cells. *J Lipid Res* 1990; 31: 113-23.
- 24 Murthy S, Albright E, Mathur SN, Field FJ. Modification of CaCo-2 membrane fatty-acid composition by eicosapentaenoic acid and palmitic acid: effect on cholesterol metabolism. *J Lipid Res* 1988; 29: 773-80.
- 25 Spector AA, Yorek MA. Membrane lipid composition and cellular function. *J Lipid Res* 1985; 26: 1015-35.
- 26 Garg ML, Thomson ABR, Clandinin MT. Interactions of saturated, n-6 and n-3 polyunsaturated fatty acids to modulate arachidonic acid metabolism. *J Lipid Res* 1990; 31: 271-7.
- 27 Dias VC, Fitzsimmons BF, Parsons HG. Eicosapentaenoic acid inhibits the arachidonic acid content of CaCo-2 microvillous membrane lipids. *FASEB J* 1990; 4: A797.
- 28 Dias VC, Borkowski JM, Parsons HG. Eicosapentaenoic acid modulates the Δ -6 and Δ -5 desaturase activity in the intestinal CaCo-2 cell line. (*abstr*) *Gastroenterology* 1991; 100: A824.
- 29 Lauritsen K, Laursen LS, Bukhave K, Rask-Madsen J. Effects of topical 5-aminosalicylic acid and prednisolone on prostaglandin E₂ and leukotriene B₄ levels determined by equilibrium in vivo dialysis of rectum in relapsing ulcerative colitis. *Gastroenterology* 1986; 91: 837-44.
- 30 Grisham MB, Granger DN. 5-Aminosalicylic acid concentration in mucosal interstitium of cat small and large intestine. *Dig Dis Sci* 1989; 34: 573-8.