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# Effect of LPS on Basal and Induced Apo E Secretion by 25-OH chol and 9cRA in Differentiated CaCo-2

**B. Ripollés Piquer,[1,2,3] H. Nazih,[1,2,3] M. Neunlist,[2,3] J.M. Huvelin,[1] and J.M. Bard[1,2,3]***

[1]Laboratoire de Biochimie Fondamentale et Appliquée, UFR de Pharmacie, Nantes, France
[2]INSERM U539, CHU, Hôtel-Dieu, Nantes, France
[3]Centre de Recherche en Nutrition Humaine, Nantes, France

**Abstract** The infection and inflammation process is associated with disturbances in lipid and lipoprotein metabolism. The apolipoprotein E (apo E) plays an important role in the lipoprotein metabolism and has been linked to inflammatory disease such as atherosclerosis and Alzheimer disease. An anti-inflammatory effect has also been suggested. The heterodimer nuclear receptor Liver-X-Receptor$_\alpha$/Retinoid-X-Receptor (LXR$_\alpha$/RXR) is considered to be a transcription factor for apo E. The aim of this study was to determine whether lipopolysaccharide (LPS) (principal component of the outer membrane Gram-negative bacteria) has an effect on apo E secretion by intestinal mucosa cells, using the Caco-2 cell line. Differentiated Caco-2 cells grown on filter inserts were incubated apically with LPS and/or 25-hydroxycholesterol (25-OH chol) and 9 cis retinoic acid (9cRA), ligands of LXR and RXR, respectively. The apical and basolateral media were separately collected. Apo E was detected by specific antibodies after protein separation by Two-dimensional nondenaturing gradient gel electrophoresis and apo E secreted in the cell culture media was measured by enzyme linked immunosorbent assay (ELISA). Apo E mRNA was analyzed by reverse transcription-polymerase chain reaction (RT-PCR). LXR$_\alpha$ and RXR mass was analyzed by Western Blot. We demonstrate here that CaCo-2 cells secrete apo E, by either apical or basolateral sides, associated with a high-density like lipoprotein, with a stoke's diameter comprised between 7.10 and 8.16 nm. We show that only apical secretion is decreased by LPS in a dose and time dependent manner. This is associated with a decrease in *apo E* gene expression contrasting with an increase of Il-8, a chemokine factor. Moreover, we demonstrate that only basolateral apo E secretion by CaCo-2 is significantly increased by 25-OH chol and 9cRA while apical secretion remains unchanged. LPS does not decrease the 25-OH chol and 9cRA mediated apo E secretion in basolateral compartment, while apical secretion is diminished under these circumstances. Our results provide evidence for the polarized secretion of apo E by intestinal epithelium. They also demonstrate that apo E secretion by CaCo-2 cell line is decreased by LPS through an LXR$_\alpha$/RXR independent signaling pathway. J. Cell. Biochem. 91: 786–795, 2004.
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**Key words:** apolipoprotein E; LPS; CaCo-2; LXR$_\alpha$/RXR

Lipopolysaccharide (LPS) is the principal component of the outer membrane of Gram-negative bacteria that are normally present in intestine. Host response to LPS induces cytokine production and then inflammation. Infection and inflammation are accompanied by alterations in lipid metabolism and in serum levels of certain proteins, so-called acute phase proteins. The alterations in lipid metabolism can be summarized by an increase in lipid and lipoprotein levels [Grunfeld et al., 1990; Krauss et al., 1990; Feingold et al., 1992; Hardardóttir et al., 1994]. This increase has been suggested to be beneficial for the host response to infection and inflammation [Gordon et al., 1996; Rauchhaus et al., 2000]. As part of plasma lipoproteins, apolipoprotein E (apo E) plays a major role in lipid metabolism [Mahley, 1988]. Local effect on major processes such as immunorregulation or peripheral nerve generation has also been described [Rensen et al., 1997; Laskowitz et al., 2000; Van Oosten et al., 2001]. In addition, it has been shown that apo E has a protective effect in Gram-negative infection [Van Oosten et al., 2001], by a direct binding to LPS [Rensen et al., 1997]. Furthermore, we have recently found a strong negative relation

*Correspondence to: J.M. Bard, Laboratoire de Biochimie Fondamentale et Appliquée, Faculté de Pharmacie, 1 rue Gaston Veil, 44093 Nantes Cedex 01, France.
E-mail: Jean-Marie.Bard@sante.univ-nantes.fr
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between C-reactive protein (CRP) and high-density lipoprotein (HDL) apo E levels in plasma [Bach-Ngohou et al., 2001].

The regulation of apo E expression has been widely studied [Duan et al., 1995; Shih et al., 2000; Brahimi et al., 2001; Grehan et al., 2001; Gueguen et al., 2001; Laffitte et al., 2001]. It has been well demonstrated that apo E transcription is directly regulated by the nuclear receptor LXR/RXR [Laffitte et al., 2001] which can be activated by oxysterols or retinoids [Willy et al., 1995; Peet et al., 1998]. This nuclear receptor has been recently associated with the acute phase response, as suggested by Beigneux et al. [2000] who have shown that LPS decreases RXR and LXR expression.

Previous studies have focused on the effects of infection and inflammation on apolipoproteins secretion in liver and macrophages and little is known on the regulation of apo E in the intestine. However, the $LXR_{\alpha}$ nuclear receptor is highly expressed in intestine [Willy et al., 1995] and it is implied in the regulation of genes involved in cholesterol metabolism [Willy et al., 1995; Murthy et al., 2002; Repa et al., 2002].

Therefore, the question of apo E secretion by intestinal cells and the influence of Gram-negative infection on this pathway remain opened.

The differentiated CaCo-2 cell line represents a unique model for investigations on the multifold aspects of the structural and functional properties of the differentiated enterocytes, including the regulation of apolipoprotein gene expression [Pinto et al., 1983; Reisher et al., 1993]. Previous studies have found that lipopolysaccharide-binding protein (LBP) is present exclusively on the apical surface of CaCo-2 cells in basal conditions [Vreugdenhil et al., 2000].

The purpose of the present study was to investigate whether differentiated CaCo-2 cells synthesize and secrete apo E in response to LPS and whether LPS has an effect on the $LXR_{\alpha}$/RXR pathway.

## MATERIALS AND METHODS

### Cell Culture

CaCo-2 cells were obtained from American Type Culture Collection (ATCC, Rockville, EEUU) and maintained in Dulbecco's modified Eagle's medium (DMEM) (Sigma, Saint Quentin Fallavier, France) containing 20% heated-inactivated fetal bovine serum (FBS) (Sigma), glutamine 2 mM (Gibco™, Invitrogen, Cergy Pontoise, France), 50 U/ml of penicillin and 50 μg/ml of streptomycin (GibcoTM, Invitrogen). Cells were grown at 37°C in a humidified incubator equilibrated with 5% $CO_2$ and 90% relative humidity. The cells were expanded in tissue culture flasks (175 $cm^2$ growth area) (Cellstar®, Greiner bio-one, VWR, Fontenay sous Bois, France). The medium was refreshed every second days until the cells reached 70–90% confluence. The cells were scrapped from the flasks by treatment with 0.25% trypsin containing 0.2% EDTA. The cells were then seeded, for individual experiments, at a density of $3 \times 10^5$ cells/well, on 24 mm polyester Transwell filter inserts with 0.4 μm pores (Costar, VWR, Fontenay sous Bois, France), that provide separation of apical and basolateral compartments (mimicking the in vivo separation of luminal and lymphatic compartments). They were allowed to grow for up to 15 days. After this time the cells were washed three-times with DMEM without FBS to remove traces of FBS. Cells were then incubated in fresh DMEM with LPS (*E. Coli* 055:B5 and *Salmonella typhosa*, Sigma) in addition to 25-hydroxycholesterol (25-OH chol) (Sigma), 9 cis retinoic acid (9cRA) (Sigma), 22(R)-hydroxycholesterol (22(R)) (Sigma), TO-901317 (Sigma), or TTNPB (Sigma). The culture medium was collected after various incubating times (24 h in most cases) and frozen at −20°C, while cells were frozen at −80°C until needed for analyses.

### Cytotoxicity Assay

Transepithelial electrical resistance across confluent CaCo-2 cell monolayer on filters was determined using the Millicell Electrical Resistance System (Millicell-ERS) (Millipore, France). This device qualitatively measure cell monolayer integrity and quantitatively measures cell confluence. To assess cell viability, LDH released into the medium was measured enzymatically according to the manufacturer's instructions with the Cytotoxicity Detection Kit (bioMerieux SA, Marcy l'étoile, France).

### Two-Dimensional Nondenaturing Gradient gel Electrophoresis of Lipoproteins

For the first dimension, 50 μl of culture medium containing secreted lipoproteins was separated by electrophoresis in 0.7% (w/v) agarose (Sigma) gel with a Tris-Tricine (Sigma) buffer (25 mM, pH 8.6), and 3 mM Calcium

Lactate (Sigma) as described by Asztalos et al. [1993]. Samples were electrophoresed at 250 V constant voltage for about 40 min. For the second dimension, the agarose gel strips from the first dimension containing the pre-separated lipoproteins were transferred to a 2–36% polyacrylamide gradient gel and sealed with the same agarose. Electrophoresis was performed using a buffer containing 90 mM Tris (Sigma), 80 mM boric acid (Euromedex, Mundolsheim, France), and 2.5 mM EDTA (Prolabo, Paris, France) (pH 8.3). Separation in the second dimension was performed at 220 V constant voltage overnight. Molecular weight standards (Amersham Pharmacia Biotech, Orsay, France) containing Thyroglobulin (669 kDa, 17 nm), Ferritin (440 kDa, 12.2 nm), Catalase (232 kDa, 9.51 nm), Lactate dehydrogenase (140 kDa, 8.16 nm), and bovine serum albumin (67 kDa, 7.10 nm) were run simultaneously with the samples. After electrophoresis, proteins were electrophoretically transferred to nitrocellulose membranes of 0.2 μm pore (Protran® Schleicher & Schuell, VWR, Fontenay sous Bois, France). Transfer was carried out for 18 h in 20 mM Tris and 150 mM glycine buffer (ph 8.4), in a Transfer Power-LID Model TE 50 (Hoefer Scientific Instruments, France) at a constant voltage (30 V) and 10°C.

Apo E in CaCo-2 cells media were identified by the use of a monoclonal mouse anti-human apo E antibody (E01) (Pasteur Institute, Lille, France) and an Horse Radish Peroxidase (HRP) labeled antibody anti-mouse IgG (Boehringer Mannheim, Roche, Meylan, France).

### Enzyme Linked Immunosorbent Assay (ELISA)

Apo E concentration in the cell culture media was determined by a highly sensitive and accurate ELISA. Briefly, microtiter plates were coated with 100 μl/well of anti-apo E antibody (Interchim, Asnieres, France) that was diluted in phosphate-buffered saline (PBS) (Gibco™, Invitrogen) at a final concentration of 5 μg/ml. After incubation at 4°C overnight, the plates were washed four-times with washing solution (PBS) and treated with blocking reagent (PBS containing bovine serum albumin 10 mg/ml (Sigma)) for 1 h at 37°C. The plates were then washed again four-times. 100 μl of each sample and of appropriately diluted standards were added to each well and the plates were incubated for 2 h at 37°C. The plates were then washed four-times with PBS and incubated with 100 μl/well of HRP-labeled secondary antibody solution (0.5 μg/ml) for 2 h at 37°C. The HRP activity was measured using 200 μl of ABTS® (Boehringer Mannheim) solution as substrate. The reaction was analyzed on a Spectra Max 190 (Molecular Devices, France) at 405 nm and at 37°C. Il-8 concentration in the cell culture media was measured according to the manufacturer's instructions with the human Il-8 BD OptEIA ELISA Kit II (BD, Le Pont de Claix, France).

### Isolation of Cellular RNA

Total cellular RNA was extracted using TRIzol® Reagent (Invitrogen) according to the method developed by Chomczynski and Sacchi [1987], and its concentration determined by absorbance at 260 nm (GENE-QUANT II, Amersham Pharmacia Biotech). The 260 nm/280 nm absorbance ratio exceeded 1.50 in all samples.

### Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and Real-Time Polymerase Chain Reaction (Q-RT-PCR)

Purified total RNA was reversely transcribed using Super Script™ II RNase $H^-$ Reverse Transcriptase enzyme (Life Technologies, Invitrogen). One microgram RNA was mixed with 1 μl (100 pM) $Pd(N)_6$ Random Hexamer 5′-Phosphate Sodium Salt (Amersham Pharmacia Biotech) and incubated for 10 min at 70°C. Then, RNA was mixed with 11.5 μl of a reverse transcription buffer containing: 7 μl sterilized water (Laboratoire Aguettant, Lyon, France), 4 μl 5× first-strand buffer (250 mM Tris-HCl (pH 8.3 at room temperature), 375 mM KCl, 15 mM $MgCl_2$), 2 μl dithiothreitol (DTT) 0.1 M, 1 μl deoxyribonucleoside triphosphate mix (10 mM each dATP, dGTP, dCTP, and dTTP at neutral pH) (dNTP) mix, and 100 U SuperScript II reverse transcriptase and incubated for 45 min at 42°C. Finally 80 μl DEPC-treated water were added to each tube. The PCR was performed on 5 μl of the prepared cDNA. Primers for human Apo E were 5′-TTCC-TGGCAGGATGCCAGGC and 5′-GGTCAGT-TGTTCCTCCAGTTC (Genosys Pampisford, UK). Primers for human β-actin, used as internal standard, were 5′GGCATCGTGATG-GACTCCG and 5′GCTGGAAGGTGGACAG-CGA (Genosys). PCR was performed with 0.5 U of Red Goldstar™ DNA Polymerase (Eurogentec, Angers, France) in an automatic DNA

thermal cycler (Perkin-Elmer, France) by adding 44.1 μl of a PCR buffer containing: 35 μl DEPC-treated water, 5 μl 10× reaction buffer (750 mM Tris-HCl (pH 8.8 at 25°C), 200 mM $(NH_4)_2SO_4$, 0.1 (v/v) Tween 20), 3 μl $MgCl_2$ 25 mM, 1 μl deoxyribonucleoside triphosphate mix (10 mM each dATP, dGTP, dCTP, and dTTP at neutral pH) (dNTP) mix and 50 pmol of each primer to the cDNA samples. An initial 3 min at 94°C followed by 35 cycles (1 min at 92°C, 1 min 50 s at 58°C, 1 min at 72°C) and an additional 10 min at 72°C were used for apo E. An initial 3 min at 94°C followed by 22 cycles (1 min at 92°C, 1 min at 67°C, 1 min at 72°C) and an additional 10 min at 72°C were used for β-actin. After amplification, 10 μl aliquots were electrophoresed in 1.5% agarose gel at 100 V for 15 min, reveled by ethidium bromide and analyzed by Quantity One (Bio Rad, Marnes la Coquette, France).

The real time RT-PCR reactions were carried out with the Kit Titanium Taq DNA polymerase (Ozyme, Clontech, St. Quentin en Yveline, France). A heat-inactivation step for 5 min at 95°C, the real time PCR reaction was performed for 40 cycles (95°C for 5 s, 68°C for 15 s, 72°C for 25 s, and 83°C for 15 s) on a Real-Time Cycler (Rotor-Gene2000, Corbett Research, France).

### Western Blot Analysis

Cells were lysed in RIPA buffer [Giri et al., 2003] (1× PBS (Gibco™, Invitrogen) 1% Igepal CA-630 ((Octylphenoxy)polyethoxyethanol) (Sigma), 0.5% sodium deoxycholate (Merck, VWR, Fontenay sous Bois, France), 0.1% SDS (Invitrogen), 10 μl/ml phenylmethylsulfonyl fluoride (10 mg/ml) (Sigma), 30 μl/ml Aprotinin (Sigma), 10 μl/ml sodium orthovanadate 100 mM (Sigma)), the homogenate was centrifuged at 10,000*g* for 10 min at 4°C. Supernatants were used for analysis of $LXR_\alpha$ and RXR protein mass. Protein contained in the total cell lysate was assayed by the BCA protein assay (Pierce Chemical Co., Interchim, Montluçon, France). Then, 40 μg of total protein were subjected to electrophoresis on a 4–10% SDS–PAGE gradient gel, transferred to nitrocellulose membranes of 0.2 μm pore (Protran® Schleicher & Schuell), and probed according to the manufacturer's recommendations with anti $LXR_\alpha$ (H-144) and anti RXR (D-20) antibodies (Santa Cruz Biotechnology, Inc., TEBU, Le Perrax en Yvelines, France). Detection of immunoreactive proteins was done by an enhanced chemiluminescence blot detection system (Santa Cruz Biotechnology).

### Statistical Analysis

Each experiment was performed in triplicate and repeated at least three-times in an independent setting. The results are expressed as mean ± SEM. Values were compared using Mann–Whitney's test, and were considered different for $P < 0.05$.

## RESULTS

### CaCo-2 Cells Secrete Apo E

First, it was studied whether apo E is secreted from apical or basolateral plasma membrane surfaces of CaCo-2 cells in basal conditions. After 24 h incubation with DMEM (without 25-OH chol, 9cRA, or LPS) the medium was harvested from both compartments and the apo E concentration was determined (Fig. 1). It was found that apo E was secreted in minor amounts (13.3 ± 2.7 ng/ml) in apical compartment, while secretion in basolateral comportment was higher (37.9 ± 2.8 ng/ml).

In order to determine whether apo E is secreted by CaCo-2 as free apolipoprotein (not complexed to lipids) or as lipoprotein, a double dimension electrophoresis was run on media as described in the "Material and Method." As shown in Figure 2 CaCo-2 cells secrete apo E as a HDL particle (Lp E) of a preβ mobility with a stoke's diameter comprised between 7.10 and 8.16 nm.

Taken together, these data indicate that both surfaces of CaCo-2 cells are able to secrete apo E as HDL particle.

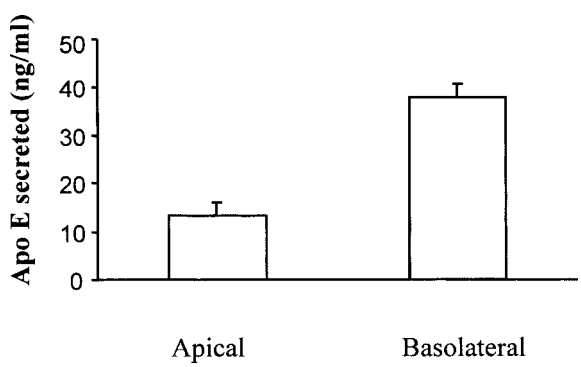


**Fig. 1.** Apolipoprotein E (apo E) secretion by CaCo-2 cells. Apo E was measured in CaCo-2 cells untreated media by enzyme linked immunosorbent assay (ELISA), as described in "Materials and Methods." Results are expressed as mean ± SEM (n = 7 in triplicates).

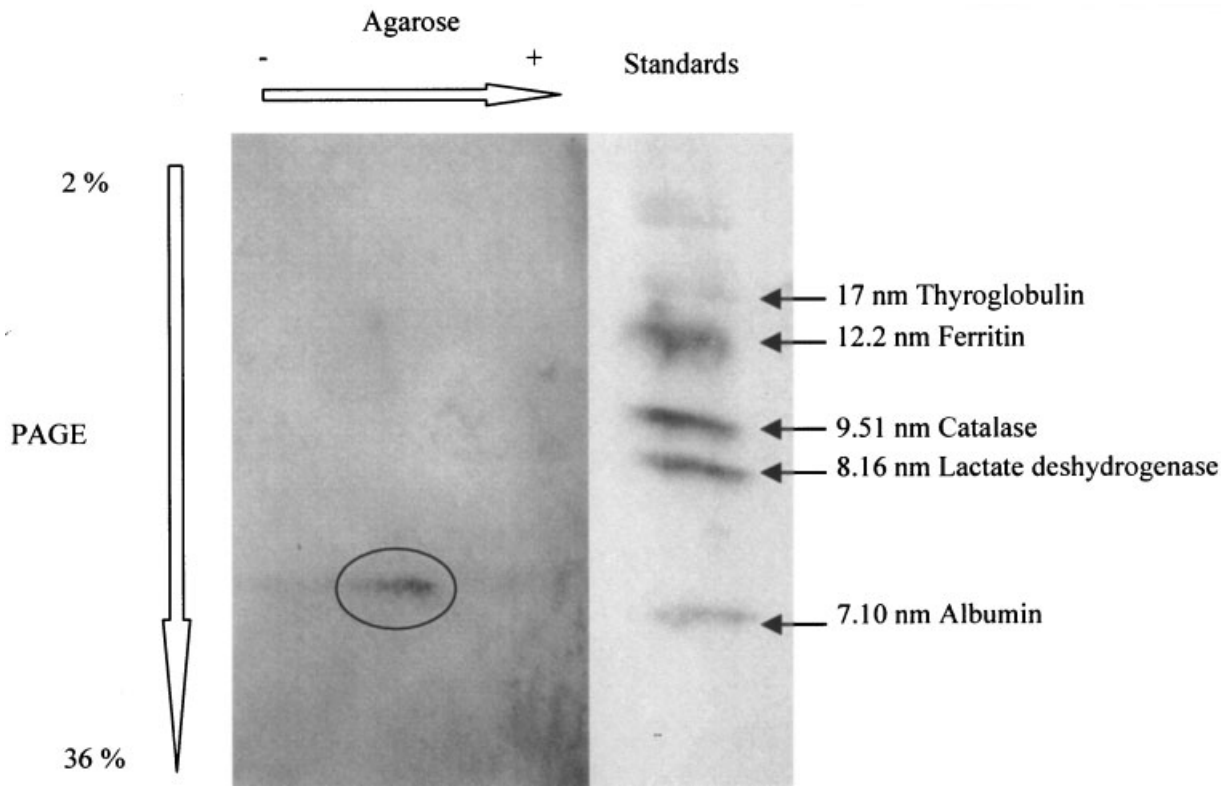


**Fig. 2.** Appearance of apo E in the second dimension after two-dimensional electrophoresis: Apical and basolateral medium were separately electrophoresed in the first dimension in 0.7% agarose followed by application of agarose strip to the top of a no denaturing 2–36% concave polyacrylamide gel and subsequently electrophoresed. The circle indicates the position of human apo E obtained after immunolocalization by a monoclonal mouse anti-human apo E antibody and an HRP-labelled antibody anti-mouse IgG.

### Cytotoxicity Test

Compared to untreated cells, the measurement of lactate dehydrogenase (LDH) activity in the culture media did not reveal any significant cytotoxicity in the presence of different concentrations of LPS (1.25–20 μg/ml) (data not shown). The monolayer integrity was shown not to be disturbed by any treatment.

### LPS Induces Il-8 Secretion

To ensure that LPS induces inflammation in CaCo-2 cells we measured the Il-8, a chemokine produced after exposure to inflammatory stimuli. Il-8 secretion was increased (by 180% in apical side and by 71% in basolateral side) after LPS stimulation of CaCo-2 cells (Fig. 3).

### LPS Inhibits the Apical Secretion of Apo E

To investigate the effect of LPS on apo E secretion, CaCo-2 cells were treated with LPS (at various concentrations) in apical side. Apo E concentration was measured in both media (apical and basolateral) at the end of incubation times.

As shown in Figure 4, LPS decreased apo E secretion in the apical side in a dose dependent manner (1.25–20 μg/ml). However no effect of LPS was observed on basolateral secretion.

To determine if the effect of LPS on apo E secretion was time dependent, we performed a kinetic study that demonstrated that LPS (10 μg/ml) inhibits apo E secretion at 4 h incubation with a maximal inhibition at 24 h incubation (data not shown). Therefore, all the following experiments were run with an incubation time of 24 h.

### LPS Decreases the Apo E Gene Expression

To determine whether the decrease in apo E concentration after LPS administration was due to a decrease in the apo E gene expression, apo E mRNA levels were analyzed by real time RT-PCR and gel-based RT-PCR. Apo E mRNA levels decreased after 24 h of LPS administration, while no effect was observed for β-actin, a

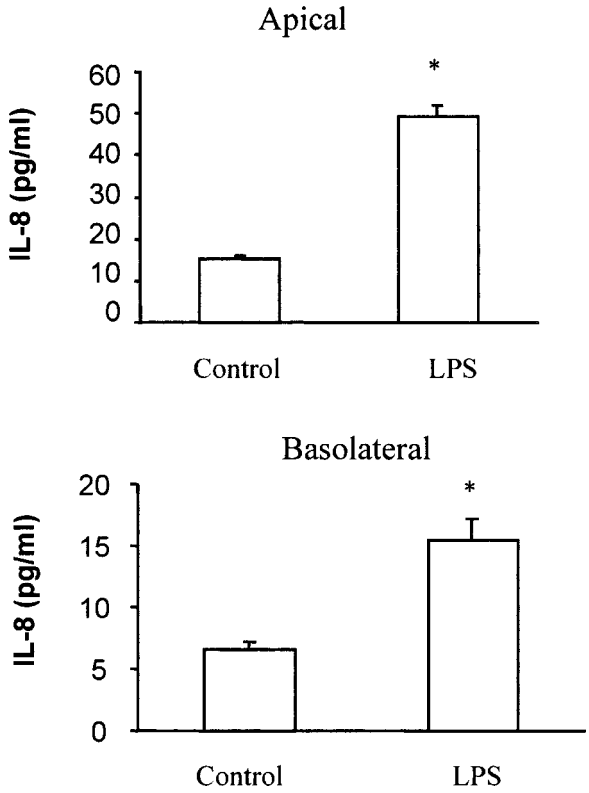


**Fig. 3.** Effect of lipopolysaccharide (LPS) on Il-8 secretion by CaCo-2 cells. Caco-2 cells were untreated (control) or incubated with LPS (10 μg/ml) for 24 h. Secreted Il-8 was measured by ELISA. Results are expressed as mean ± SEM (n = 3 separate experiments in triplicates); *, significant difference from control ($P < 0.05$).

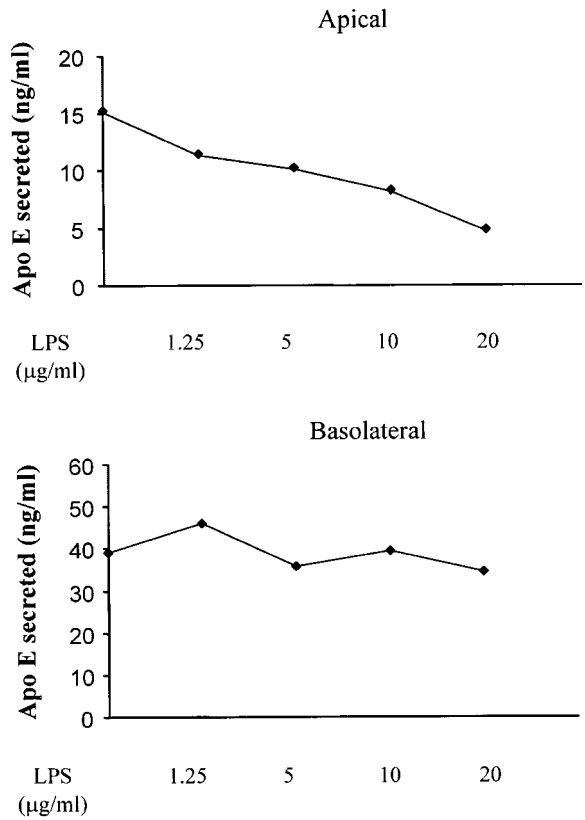


**Fig. 4.** Effect of LPS on apo E secretion by CaCo-2 cells. Caco-2 cells were untreated (control) or incubated with increasing concentrations of LPS (1.25, 5, 10, and 20 μg/ml) for 24 h. Secreted apo E was measured by ELISA as described in "Materials and Methods." Results are expressed as mean of triplicate values. This is a representative of two independent experiments.

housekeeping gene (Fig. 5). This indicates that this decrease is almost partially due to a decrease in apo E gene transcription.

### 25-OH chol and 9cRA Activate the Basolateral Secretion of Apo E

25-OH chol is among oxysterols known to induce apo E secretion [Gueguen et al., 2001]. It binds and activates the nuclear receptor LXR which was recently shown to be a key regulator of apo E gene expression in macrophages and adipocytes [Laffitte et al., 2001]. LXR regulates target genes by binding to specific promotor response element (LXREs) in association with its obligate heterodimerization partner, Retinoid X Receptor (RXR), the receptor of 9cRA [Costet et al., 2000; Ohama et al., 2002].

To determine whether 25-OH chol and 9cRA also play a role in enterocytes apo E secretion, we examined their influence on apo E secretion in differentiated CaCo-2 cells. Compared with basal conditions, cells treated with 25-OH chol or 9cRA exhibited an increase in apo E secretion (data not shown). As shown in Figure 6, when both compounds were added together, there was also an increase in apo E secretion, without any synergistic induction. More specific ligands of LXR (22(R) and TO-901317) were also studied. Consistent with 25-OH chol data treatment of CaCo-2 cells with either 22(R) and TO-901317 led to an increase in apo E secretion. However TTNPB, a specific ligand of RAR did not modulate apo E secretion (data not shown).

These results suggest that the apo E secretion by CaCo-2 cells is at least partly regulated by $LXR_{\alpha}$/RXR nuclear receptor.

### Effect of LPS on Apo E Secretion Induced by 25-OH chol and 9cRA Activation

As shown in Figure 6 LPS decreases significantly the apo E apical secretion even in the presence of the 25-OH chol and 9cRA. Co-

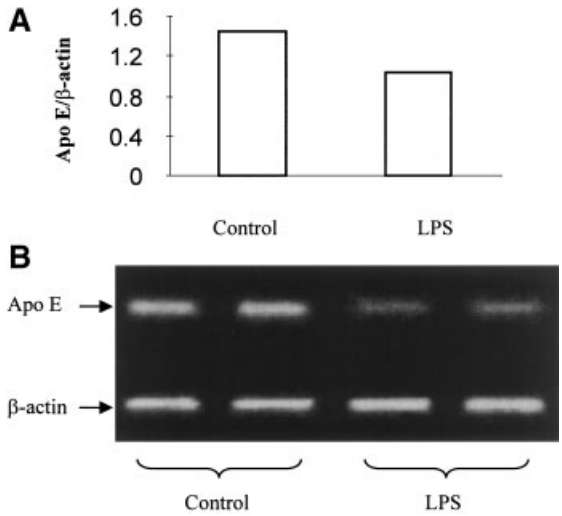


**Fig. 5.** Effect of LPS on apo E gene expression in Caco-2 cells measured by reverse transcription-polymerase chain reaction (RT-PCR). Real-time polymerase chain reaction (Q-RT-PCR) and gel based RT-PCR assay for apo E mRNA. **A**: Real time RT-PCR assay for apo E/β-actin mRNA ratio (mean of triplicate values) in Caco-2 cells untreated or incubated with LPS. This is a representative of two independent experiments. **B**: Gel-based RT-PCR after 35 cycles showing apo E and β-actin mRNA in CaCo-2 cells with or without treatment with LPS (10 μg/ml) (image used after UV visualization of ethidium bromide-stained bands). A representative gel of seven experiments is shown.

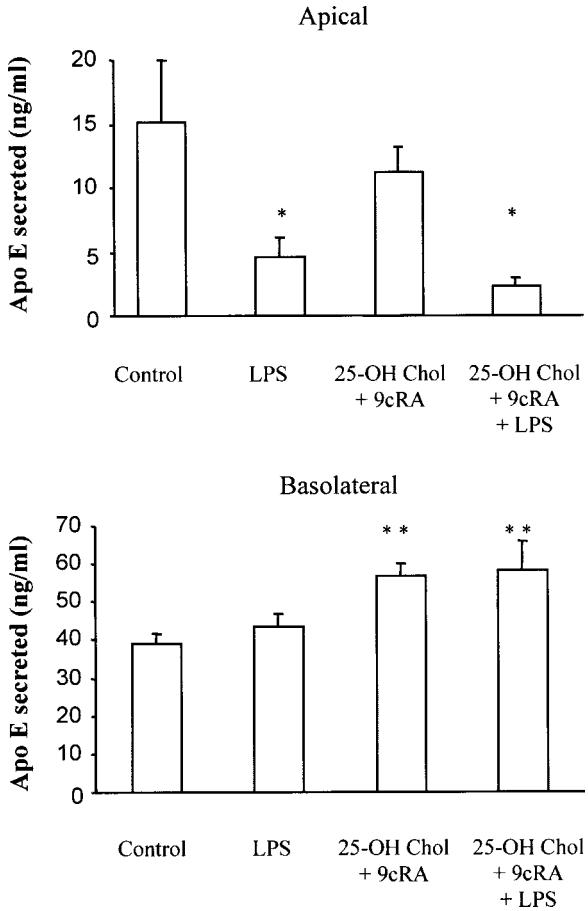


**Fig. 6.** Effect of LPS on induced apo E secretion by 25-hydroxycholesterol (25-OH chol) and cis retinoic acid (9cRA) in CaCo-2 cells. CaCo-2 cells were untreated (control) or incubated with LPS (10 μg/ml), 9cRA and 25-OH chol (10 μM) or with 9cRA and 25-OH chol (10 μM) and LPS (10 μg/ml) for 24 h. Secreted apo E was measured by ELISA as described in ''Materials and Methods.'' Results are expressed as mean ± SEM. (n = 4 in triplicates); *, significant difference from control ($P < 0.03$); **, significant difference from control ($P < 0.002$).

incubation of cells with LPS and 25-OH chol and 9cRA did not alter the increasing effect of 25-OH chol and 9cRA on apo E secretion at the basolateral side (Fig. 6). Taken together these results show that both phenomenon, increased apo E basolateral secretion by 25-OH chol and 9cRA and decreased apo E apical secretion by LPS are independent.

Figure 7 shows results from three experiments investigating the effect of LPS activation on $LXR_{\alpha}$ and RXR mass. Cells were incubated for 24 h with LPS. Following the incubation, $LXR_{\alpha}$ and RXR separated by SDS–PAGE were estimated by immunoblotting using specific antibodies against $LXR_{\alpha}$ and RXR. Compared with untreated cells, LPS did not decrease the amount of $LXR_{\alpha}$ and RXR.

## DISCUSSION

The effect of inflammation and infection on apo E expression and secretion has been widely studied, and the findings are controversial [Duan et al., 1995; Hua et al., 1998; Brahimi et al., 2001; Gueguen et al., 2001]. On the other hand a protective effect against Gram-negative infection has been suggested [Van Oosten et al., 2001]. It has been shown that the secretion of other apolipoproteins such as apo A-I and apo B, is decreased in response to cytokines from both surfaces of CaCo-2 cells [Vreugdenhil et al., 2000]. To our knowledge, the current study is the first to evaluate the effect of LPS on apo E secretion by CaCo-2 cells. First, we demonstrated that CaCo-2 cells secrete apo E on both sides. Several lines of evidence were obtained: 1. detection by ELISA in apical and basolateral media; 2. Western blot in apical and basolateral media (data not shown); 3. characterization of secreted lipoproteins by 2D-PAGE on apical and basolateral media; 4. analysis of mRNA by

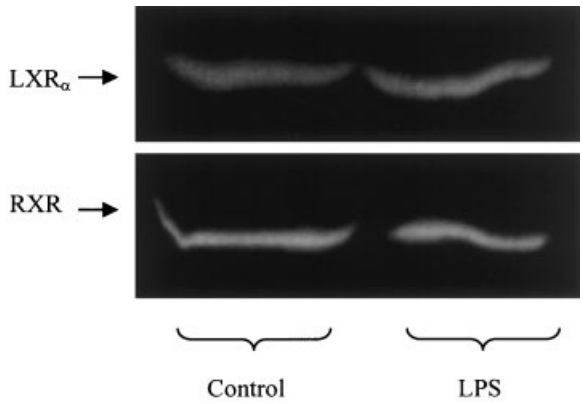


**Fig. 7.** Effect of LPS on $LXR_\alpha$/RXR mass. CaCo-2 cells were incubated with LPS (10 μg/ml) for 24 h, cells were lysed and total proteins isolated as described in ''Material and Methods.'' Proteins were separated by SDS–PAGE and transferred to nitrocellulose. Detection of $LXR_\alpha$ and RXR was possible using polyclonal anti $LXR_\alpha$ and anti RXR antibodies. A representative immunoblot of three independent experiments is shown.

RT-PCR; 5. the kinetic dependence of its appearance; 6. its induction by 25-OH chol, 22(R), and TO-901317, ligands of LXR, and 9cRA, ligand of RXR. This apo E is secreted associated with lipoproteins exhibiting the characteristics of HDL by either the apical or the basolateral side. This is in agreement with previous results showing that the majority of the human apo E secreted from the transfected McARH7777 cells, a rat hepatoma cell line was associated with HDL particles [Reardon et al., 1998].

The results of the present study strongly implies that a substantial portion of the apo E under basal conditions is released into apical medium. This has already been recognized for other apolipoproteins (AI and B) [Vreugdenhil et al., 2000] for the same cell line. A further study to dissect the process of vectorial secretion might therefore provide more insights into some of the mechanisms that regulate intracellular membrane traffic.

Second, we demonstrated that in basal conditions LPS significantly decreased the synthesis and secretion of Apo E in a dose and time dependent manners.

Remarkably, apical exposure of cells to LPS resulted in a decrease in apo E apical secretion whereas basolateral secretion remained unchanged. Further studies are needed to determine by which mechanism apical LPS stimulation may interfere with apical apo E secretion. Recently, Laffitte et al. [2001] demonstrated that nuclear receptors LXR and oxysterols ligands are key regulators of apo E expression in both macrophages and adipose tissue. They show that LXR/RXR heterodimers regulate apo E transcription through direct interaction with LXR response element (LXRE). These nuclear receptors are highly expressed in the intestine [Willy et al., 1995]. In our study, we show that only basolateral apo E secretion by CaCo-2 cells is significantly increased by 25-OH chol, 22(R), and TO-901317, ligands of LXR, and 9cRA, ligand of RXR while apical secretion remained unchanged.

In recent studies, [Costet et al., 2000; Venkateswaran et al., 2000; Laffitte et al., 2001; Repa et al., 2002] it has been demonstrated that the ATP-binding cassette (ABC) family of transporter proteins, are direct transcriptional targets of LXRs. ABCA1 have been suggested to be involved in lipid efflux [Bortnick et al., 2000]. Indeed, in lipid-loaded macrophages, induction of ABCA1 expression by LXR ligands similarly promotes cholesterol transfer to extra-cellular acceptors such as apo A-I [Laffitte et al., 2001]. Other studies suggest a central role for LXR signaling pathways in the control of macrophages cholesterol efflux through the coordinate regulation of apo E and ABC expression [Laffitte et al., 2001; von Eckardstein et al., 2001]. Two recent studies have confirmed that ABCA1 in CaCo-2 cells is localized in basolateral compartment [Murthy et al., 2002; Ohama et al., 2002]. Together with previous studies, our observations support the hypothesis that $LXR_\alpha$/RXR heterodimers lead to the coordinate apo E and ABC transporters regulation and secretion of apo E to basolateral compartment.

To determine if LPS has an effect on apo E secretion induced by 25-OH chol and 9cRA we performed co-administration of LPS, 25-OH chol, and 9cRA. Interestingly, LPS did not decrease the 25-OH chol and 9cRA mediated apo E secretion in basolateral compartment. However, under these circumstances apical secretion was decreased to a similar extent than what was observed with LPS alone. This suggests that the effect of LPS on apo E secretion on the apical side does not interfere with the $LXR_\alpha$/RXR pathway. This hypothesis seems to be supported by the observed lack of $LXR_\alpha$ and RXR mass modification under stimulation of CaCo-2 cells by LPS.

In summary, our results provide evidence for the vectorial secretion of apo E by intestinal epithelium, a phenomenon that has not been described before. They also demonstrate that

the apo E secretion by CaCo-2 cells is decreased by LPS, a direct mediator of the effect of Gram-negative infection. Further studies are needed to clarify the mechanisms by which LPS decreases apo E secretion.

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