High-Density Lipoproteins Differentially Modulate Cytokine-Induced Expression of E-Selectin and Cyclooxygenase-2

Gillian W. Cockerill, Jeremy Saklatvala, Simon H. Ridley, Helen Yarwood, Norman E. Miller, Barbaros Oral, Saro Nithyanathan, Graham Taylor, Dorian O. Haskard

Abstract—Atherogenesis is a multifactorial chronic inflammatory disease in which low plasma levels of HDLs are a strong predictor of the condition. Although the mechanism of protection by HDLs is not precisely known, HDLs have been shown to influence many of the events involved in the development of atherosclerosis. Previously we have shown that HDLs inhibited the cytokine-induced expression of adhesion molecules (E-selectin, VCAM-1, and ICAM-1) by endothelial cells (ECs). As the complete transcriptional regulation of all 3 genes requires the NF-κB family of transcription factors, we examined the effect of HDLs on activation of NF-κB. We also investigated the effect of HDLs on 2 other cytokine-induced genes, granulocyte-macrophage colony-stimulating factor (GM-CSF) and cyclooxygenase (Cox-2; prostaglandin H₂ synthase, EC 1.14.99.1). E-selectin expression in response to tumor necrosis factor-α (TNFα) was, as expected, inhibited in ECs that had been preincubated with HDLs. However, the level of secretion of GM-CSF in the same cultures was no different from control. In a similar manner, although HDLs had no effect on steady-state mRNA levels of GM-CSF, the levels of E-selectin were significantly inhibited by HDLs. In transient cotransfection experiments we found that HDLs inhibited the cytokine-induced expression of a reporter gene driven by the E-selectin proximal promoter (−383 to 80) but had no effect on the expression of a reporter gene driven under the control of the proximal promoter of GM-CSF (−627 to 28). As would be predicted from this differential response, HDLs did not influence the nuclear translocation or DNA binding of NF-κB, or alter the kinetics of degradation and resynthesis of the inhibitory protein IκBα. We found that HDLs synergized with cytokine to enhance the expression of Cox-2 and induce the synthesis of its main EC product, prostacyclin (PGI₂), a potent inhibitor of platelet and leukocyte functions. In conclusion, HDL induces an antiinflammatory phenotype in cytokine-induced ECs, synergizing with cytokine to induce elevation of Cox-2 in addition to inhibiting adhesion molecule expression. Our studies show that these differential effects are mediated in a manner that is likely to be independent of NF-κB per se. (Arterioscler Thromb Vasc Biol. 1999;19:910-917.)

Key Words: inflammation ■ coronary artery disease ■ prostacyclin ■ granulocyte-macrophage colony-stimulating factor

Coronary artery disease remains one of the major causes of death in the Western world today. A great deal of epidemiological research shows a strong inverse correlation between the concentration of plasma HDL and the frequency of coronary artery disease. More direct evidence of the protective effects of HDL comes from studies in which elevation of HDL in animal models has led to a dramatic protective effect against early atherosclerotic fatty streak formation. It is not yet known whether the protective effects of HDL relate to their role in reverse cholesterol transport or to mechanisms unrelated to their lipid transport function.

Normal endothelium is one that maintains a nonadhesive, nonthrombogenic, and nonproliferative interface of cells that separate the blood from the underlying tissues. The phenotype of the endothelium changes in response to certain inflammatory mediators, such as tumor necrosis factor-α (TNFα) and interleukin-1β (IL-1β), to one that expresses and secretes several adhesion molecules and chemoattractants capable of promoting the recruitment of leukocytes across the endothelial barrier. Many cytokine-stimulated genes are regulated at the transcriptional level by transcription factors of the NF-κB family. Normally, NF-κB members are confined to the cytoplasm through association with inhibitory proteins of the IκB family. IL-1β or TNFα leads to the phosphorylation and degradation of the IκB proteins, resulting in the release, nuclear translocation, and DNA binding of NF-κB, with subsequent transcriptional activation of respon-
sive genes. In many instances this process is transitory, as NF-κB stimulates the transcription and resynthesis of IkBα.23–25

There is considerable evidence for the importance of leukocyte recruitment into the arterial intima, both for the development of atherosclerosis and for the maintenance of the mature plaque. The earliest observable cellular event in the genesis or development of atheroma is the binding of leukocytes to the endothelium.26,27 Furthermore, there are now several studies demonstrating the presence on endothelium of IL-1 or TNF-inducible adhesion molecules, such as P-selectin, E-selectin, VCAM-1, and ICAM-1, both in animal models of atherosclerosis and in human atherosclerotic tissue.28–32 In a previous study the possibility was explored that those using native HDL.

**Methods**

**Cell Culture**

HUVECs were isolated as described previously.33 All cells were plated onto 1% gelatin-coated culture plastic in medium 199 with Earle’s salts (ICN Biomedicals Inc) supplemented with 20% FCS (GIBCO), 2 mmol/L glutamine, penicillin and streptomycin, 50 μg/mL EC growth supplement (Sigma), and 50 ng/mL heparin (Sigma). In typical experiments, confluent HUVECs were incubated for 16 hours with native or reconstituted HDL (1 mg/mL apoAI), after which TNFα or IL-1α (1 ng/mL) was added to the culture medium for a further period before ending the experiment. From using this concentration of cytokines, no loss of cell viability was observed.

**Isolation of HDL**

Blood from normal healthy volunteers, <40 years of age, was collected in Vacutainers containing K-EDTA as the anticoagulant (Beckton Dickinson). Plasma was separated by spinning at 2000 rpm for 20 minutes at 4°C. HDLs were then purified by sequential ultracentrifugation in the 1.07 to 1.21 g/mL density range, according to the previously described method.34 Resulting HDLs were then dialysed against 4 changes of PBS and filter-sterilized by using a 0.2-nm Minisart microfilter (Sartorius). HDL isolated in the presence of EDTA loses paraoxonase,35 hence effects observed in these studies are independent of this enzyme.

**Analysis of HDL**

Concentrations of apoAI and apoB were determined by immunoturbidimetric methods, using a Cobas-Fara centrifugal analyzer (Roche Diagnostic) with commercially available antibodies (Boehringer Mannheim). HDL particle size was evaluated by electrophoresis through 3% to 30% nondenaturing gradient gels (Pharmacia). All preparations of HDL contained 2 main populations: 1 with a Stokes diameter of 10.4 nm (HDL 3a). HDL preparations contained no contaminating particles of the size range of LDL and there was no apoB detectable by immunoturbidimetric analysis. All preparations used were endotoxin free, as determined by the limulus lysate microassay,36 and the inability of native HDL to elevate basal adhesion molecule expression on HUVECs (Figure 1).

**Reconstituted HDL**

The reconstituted HDLs used in this study were kindly provided by the Swiss Red Cross, Blood Transfusion Service, Bern, Switzerland. The particles, containing apoAI as the sole protein, and soybean phosphatidylcholine as the sole phospholipid, were prepared by using cholate dialysis according to the reported method.37 Reconstituted discoidal HDL particles were used in parallel experiments to those using native HDL.

**Flow Cytometry**

HUVECs were plated at confluence in 24-well plates (2 × 10^4 cells/400 μL per well), and incubated with HDL and TNFα (as above). Cells were then harvested with trypsin and washed in ice-cold PBS. Surface expression of E-selectin was then determined by resuspending the cells in 50 μL of anti-E-selectin monoclonal antibody38 for 45 minutes at 4°C. Cells were then washed in PBS.
containing 0.02% sodium azide and 5% newborn calf serum (Gibco) at 4°C and incubated for 45 minutes at 4°C in 0.5 µL of wash buffer containing FITC-labeled rabbit anti-mouse Ig (DAKO). After further washing, the cells were fixed with 2.5% formaldehyde. Expression of E-selectin was measured by using a Coulter Epics Profile II flow cytometer, counting 10⁴ cells per sample. Controls included an isotype-matched irrelevant antibody and no primary antibody.

**GM-CSF Enzyme-Linked Immunosorbent Assay**

GM-CSF protein concentrations in EC culture supernatants were determined by using a human GM-CSF ELISA kit (R & D Systems Ltd) according to the manufacturer’s recommendations.

**Northern Analysis**

Total RNA was prepared as described by Chomczynski and Sacchi. Equal aliquots of total RNA (10 µg/lane) were then pelleted and lysed in 50 µL of lysis buffer (50 mM Tris-Cl, 1% NP-40, 0.25% deoxycholate, 5 mM/100 mM EDTA, 1 mM NaCl, 10 mM NaF, 5 mM/100 mM leupeptin, 100 µM aprotinin, and 1 mM/100 PMSF (Sigma)) ice on 15 minutes for ice. Debris was then removed by centrifuging the lysate for 5 minutes at 4°C and the supernatants stored at −70°C until use. Protein concentration was estimated by using the Bio-Rad BCA kit (Bio-Rad). Protein was separated by running 10 µg of protein lysate in a 15% SDS-PAGE, using a Protein Mini gel apparatus. Proteins were transferred to a nylon (Millipore) membrane, according to the manufacturer’s recommendations, using the Protein Mini gel wet transfer apparatus. Filters were then blocked overnight in 5% milk solid in PBS and washed once for 15 minutes in PBS with 0.05% Tween (Sigma) and incubated on ice in 15 minutes for PBS containing 0.5% milk solid for 1 hour. After extensive washing (5/15 minutes in PBS containing 0.05% Tween), the protein–antibody complex was identified by incubating the filter for 1 hour in a 1:10,000 dilution of an HRP-conjugated goat anti-rabbit immunoglobulin (Sigma) in hybridizing buffer. After further washes (5/15 minutes with PBS containing 0.05% Tween, and 2×15 minutes with PBS only), the filter was treated with chemiluminescence reagent according to the manufacturer’s recommendations (ECL) and exposed to Kodak BIOMAX film. Equivalent loading was monitored by protein staining the filter using Ponceau S.

**Prostacyclin Synthesis Assay**

The stable hydrolysis product of prostacyclin, 6-oxo-prostaglandin F₁α (6-oxo-PGF₁α), was measured by using an immunoabsorbance assay (Cayman Chemicals). According to the manufacturer’s recommendation. The identity of the antigen quantified by this assay was confirmed by immunoabsorbance analysis of fractions of supernatant separated by HPLC (Waters St Quentin en Yvelines) Model 510 with an Altex ultosphere column (Beckman). The mobile phase was composed of 10% acetonitrile (HPLC Grade, Merck), 5 mM/100 mM phosphoric acid (solvent 1) and 50% acetonitrile, and 5 mM/100 mM phosphoric acid (solvent 2). The wavelength detection monitored was 204 nm.

**Electrophoretic Mobility Shift Assay (EMSA)**

EMSA were performed as previously described. In brief, 5 µg of nuclear extract with 0.2 ng of 32P-labeled probe, 2 µg of poly (dI–dC) (Promega) in a binding buffer (30 mM/100 HEPE, pH 7.9, 16 mM/100 KCl, 0.5 mM/100 DTT, 13% glycerol, 8 µg/mL leupeptin, and 8 µg/mL aprotinin) in a final volume of 18 µL for 30 minutes at room temperature. Samples were fractionated by electrophoresis through a 4% low-salt (0.5x) 89 mM/100 Tris, 89 mM/100 borate, and 2 mM/100 EDTA, pH 8.0) PAGE and visualized by autoradiography using Kodak BIOMAX.

**Nuclear Extract Preparation**

Nuclear extracts were made according to the method of Dignam et al., with minor modification. Confluent HUVEC monolayers were treated as indicated in the text, washed twice with ice-cold PBS, and then harvested by scraping. Cells were then pelleted at 1000 rpm for 3 minutes in a Beckman CS-6R benchtop centrifuge. Supernatants were discarded and the pellet resuspended by the rapid addition of 5 packed cell volumes of hypotonic buffer (10 mM/100 HEPE, pH 7.9, 1.5 mM/100 MgCl₂, 10 mM/100 PMSF, and 0.5 mM/100 DTT). After immediately pelleting the cells at 1000 rpm for 5 minutes at 4°C, the cells were resuspended in 3 packed cell volumes of hypotonic buffer. Cells were then incubated for 15 minutes on ice, during which cell lysis was monitored by viewing aliquots of the reaction with an inverted microscope with Hoffman optics. Nuclei were then pelleted at 4000g, and the supernatant containing cytoplasmic factors carefully removed. The nuclei pellet was then resuspended in half a packed nuclei volume of low-salt buffer (20 mM/100 HEPE, pH 7.9, 40 mM/100 NaCl, 25% glycerol, 1.5 mM/100 EDTA, 0.2 mM/100 PMSF, 0.5 mM/100 DTT, 8 µg/mL leupeptin, and 8 µg/mL aprotinin) followed by 0.5 packed nuclei volume of high-salt buffer (as for low salt, with 1.2 mM/100 KCl), added slowly while stirring the nuclei with a pipette tip. The extraction mixture was then agitated on a shaking platform for 30 minutes at 4°C and nuclear extract recovered by spinning down the nuclei at 13 000 rpm in an Eppendorf microfuge at 4°C. Supernatants containing the nuclear extracts were then recovered and stored at −70°C before use. Protein concentration of the nuclear extracts was measured by using the Bio-Rad BCA kit (Bio-Rad).

**Purification and Labeling of the Oligonucleotide Probe**

NF-κB–specific oligonucleosome duplexes had the following sequence with complimentary single-stranded regions at each end shown in lower case for the upper strand only: E-selectin NF-κB, aatCGTTTTGATG-GCCATTGGGATTTCCTCTTTACTGGATGTG; and Igκ NF-κB, AACAGGGGACCTTTCGAGGCCCATCT.

The oligonucleotides were labeled by end-filling, using DNA polymerase I (Klenow) fragment (Promega) (E-selectin NF-κB) or kinase end-labeling (Igκ NF-κB) and gel-purified on a low-salt 7.5% non-denaturing PAGE.

**Plasmids**

The luciferase reporter gene plasmid pGM-Luc contains a 655-bp fragment of the human GM-CSF promoter (−627 to 28) upstream of the luciferase gene (Promega). The chloramphenicol acetyl transferase (CAT) reporter gene plasmid pCAT-Elsu contains a 463-bp fragment of the human E-selectin promoter (−383 to 80) upstream from the CAT gene (pCAT-Basic, Promega). The β-galactosidase vector pCMVβ (Clontech) containing the constitutively active cyto-megalovirus (CMV) promoter was used as a cotransfectant to correct for variations in transfection efficiency. All of the expression plasmid vectors were purified by CsCl equilibrium centrifugation. The luciferase reporter gene plasmid pNF-κB3 includes 3 concatenated sequences of the E-selectin NF-κB consensus sequence.

**Endothelial Cell Transfection**

HUVECs were cotransfected with reporter genes using electroporation, according to a method previously described. In brief, 10⁷ confluent HUVECs were trypsinized into suspension and washed twice in HEPES-buffered saline. Cells were finally resuspended in 300 mL of HEPESE-buffered saline containing 50 µg of pGM-Luc, 50 µg of pCAT-Elsu, and 5 µg of pCMVβ plasmids, then shocked in a 4-mm electroporation cuvette (Bio-Rad) at 250 V, 960 mJ, giving a time constant of 20 msec. Cells were then immediately resuspended in normal growth medium and plated into 6×60-mm culture dishes precoated with 1% gelatin (Sigma). Growth medium was changed.
by guest on July 9, 2017 http://atvb.ahajournals.org/ Downloaded from

The NF-κB activation, then it is likely that most genes known to require

were normalized to manufacturer’s recommendations (Boehringer Mannheim and experiments, using HDL from 3 separate donors. E-selectin, GM-CSF, and GAPDH. Data are representative of 3 and analyzed by northern analysis by using cDNA probes to E-selectin, GM-CSF, and GAPDH. Data are representative of 3 experiments, using HDL from 3 separate donors.

Luciferase, CAT, and β-Galactosidase Assays

Transfected cells were harvested and lysed in reporter lysis buffer (Promega), according to manufacturer’s recommendations, which allow extracts to be analyzed for luciferase, β-galactosidase, and CAT. Luciferase activity was assayed by using the Promega kit according to the manufacturer’s specifications, measuring photons using a Turner Design Model 20/20 luminometer. CAT and β-galactosidase activity was measured using kits, according to the manufacturer’s recommendations (Boehringer Mannheim and Promega).

Results

The data shown are representative experiments where the HDL preparations used are native heterogeneous samples isolated from different donors as described in Methods. In parallel experiments, the use of reconstituted discoidal HDL particles, in which apoAI is the sole apolipoprotein and phosphatidylcholine is the sole phospholipid, gave similar results (data not shown).

If HDLs were able to inhibit cytokine-induced adhesion molecule expression by inhibiting some element of NF-κB activation, then it is likely that most genes known to require the NF-κB family of transcription factors should be inhibited by treatment with HDLs. We therefore examined the effect of HDL treatment on a range of cytokine-inducible genes. Preincubation of HUVECs with HDLs resulted in an inhibition of cytokine-induced adhesion molecule expression, as has been shown previously, although clearly having no effect on GM-CSF secretion (Figure 1) or steady-state mRNA level (Figure 2).

Although the proximal promoters of both E-selectin and GM-CSF have a major requirement for the NF-κB family of transcription factors, the GM-CSF gene is also regulated by a distal enhancer region, located at 716 to 625 bp upstream from the transcriptional start site of GM-CSF, controlled via a cyclosporin-sensitive nuclear factor of activated T cells binding site. Activation of nuclear

factor of activated T cells requires both the elevation of Ca2+ and mobilization of protein kinase C. As HDLs have been shown to be capable of inducing both these responses, it was possible that the lack of effect on GM-CSF synthesis is by induction of the calcium-sensitive distal enhancer region, which could mask any inhibition of the proximal promoter region. To overcome this consideration, we compared the effect of HDLs on the TNF-inducible expression of reporter genes controlled by the E-selectin and GM-CSF proximal promoters in transient transfection assays in HUVECs. Whereas HDLs had no effect on the stimulation of luciferase activity under the control of the GM-CSF promoter (--627 to 28 bp) in a CAT reporter plasmid, the GM-CSF promoter (--388 to 80 bp) in a luciferase reporter plasmid and the β-galactosidase reporter gene, cells were incubated either with or without HDL (1 mg/mL apoAI) for 16 hours followed by a 9-hour incubation with TNFα (10 ng/mL) in the presence (HDL,TNF) or the absence (TNF) of HDL (1 mg/mL apoAI). Levels of gene activation were measured and compared with untreated cells (NIL). Levels of CAT (a) and luciferase activity (b) are expressed as relative values normalized to levels of β-galactosidase. Values represent the mean and SD and are representative of 6 similar experiments, using HDL from 6 separate donors. Differences between means were evaluated by using Student’s unpaired t test. *P, not significant; **P<0.005.

Figure 3. HDLs inhibit the TNFα-induced expression of the CAT reporter driven by the E-selectin promoter but have no effect on the expression of a luciferase reporter driven by the proximal promoter of GM-CSF. Forty-eight hours after cotransfection of confluent ECs with the E-selectin promoter (~627 to 28 bp) in a CAT reporter plasmid, the GM-CSF promoter (~388 to 80 bp) in a luciferase reporter plasmid and the β-galactosidase reporter gene, cells were incubated either with or without HDL (1 mg/mL apoAI) for 16 hours followed by a 9-hour incubation with TNFα (10 ng/mL) in the presence (HDL,TNF) or the absence (TNF) of HDL (1 mg/mL apoAI). Levels of gene activation were measured and compared with untreated cells (NIL). Levels of CAT (a) and luciferase activity (b) are expressed as relative values normalized to levels of β-galactosidase. Values represent the mean and SD and are representative of 6 similar experiments, using HDL from 6 separate donors. Differences between means were evaluated by using Student’s unpaired t test. *P, not significant; **P<0.005.
isoforms of this enzyme, Cox-1, which is present in several cell types and is constitutively expressed and relatively stable,56,57 and Cox-2, which is the inducible form.56–58 As shown in Figure 7, not only could HDL alone stimulate Cox-2, but HDL synergized with TNFα and IL-1β to augment this increase. Consistent with the findings of others (Dr...
HDLs synergize with the cytokines TNFα and IL-1β to elevate the level of Cox-2 expression in ECs. Western blot analysis of whole cell lysates (10 μg/lane) prepared from confluent ECs preincubated with HDL (1 mg/mL apoAI) alone for 8 hours or preincubated with HDL for 4 hours and then treated with either TNFα (1 ng/mL) or IL-1β (1 ng/mL) and HDL for a further 4 hours. The lanes have been probed with antibody against Cox-2 (Biogenics Ltd). Results are representative of 3 experiments, using HDL from 3 separate donors.

Discussion

In this study, we have shown that in addition to inhibiting the expression of adhesion molecules for leukocytes, HDL can synergize with TNFα and IL-1β to induce levels of Cox-2 and the secretion of prostacyclin. The in vitro EC phenotype induced by HDL is therefore one of reduced surface adhesiveness for leukocytes, together with increased secretion of a factor that can inhibit platelet and leukocyte activation and induce vasodilation. This phenotype might be expected to contribute to the in vivo mechanisms by which this multipotent lipoprotein reduces the risk of atherosclerosis.

Our findings that HDL have differential effects on expression of E-selectin, GM-CSF, and Cox-2 in response to IL-1β and TNFα stimulation challenge that HDL has a general inhibitory effect on the NF-κB pathway. All 3 genes have been shown to have a requirement for the NF-κB family of transcription factors for full cytokine-mediated expression.90–91 The mechanism by which HDLs modulate cytokine-mediated E-selectin expression is at least mediated at the transcriptional level, as we also observed inhibition of steady-state mRNA level and repression of a reporter gene driven by a full E-selectin proximal promoter. Furthermore, by using a reporter construct containing 3 tandem E-selectin NF-κB binding domains, we were unable to show inhibition of expression by HDL treatment. Consistent with this, we were unable to detect any inhibition by HDL of TNF-mediated NF-κB nuclear translocation or DNA binding, or a difference in the kinetics of degradation and resynthesis of mRNA, a process known to be NF-κB dependent.23–25 These data therefore suggest that HDL primarily influences transactivating factors other than those of the NF-κB pathway itself. Similar conclusions may be drawn from the recent work of Wolle et al.62 who have shown that flavone (PD 09806) inhibits VCAM-1 expression by human aortic ECs but does not affect the binding or translocation of NF-κB or inhibit the transactivation of a reporter construct containing 2 tandem VCAM-1 NF-κB sites. Further deletion analysis of the E-selectin promoter is now required to establish the specific domains involved in HDL-mediated inhibition.

The original observation that HDL could induce EC synthesis of prostacyclin was attributed to the ability of the lipoprotein to provide the substrate, arachidonic acid.53–55,62,63 Our findings that HDL synergizes with IL-1β and, to a lesser extent TNFα, to increase Cox-2 protein levels and prostacyclin production is novel, and pertinent to atherogenesis, in which both cytokines are likely to play a role.64 Our studies offer an additional mechanism whereby prostacyclin is elevated by HDL, but do not preclude the possibility that HDL may also supply exogenous substrate for the synthesis of the eicosanoid. However, as reconstituted discoidal HDL particles, containing only apoAI as the sole protein and phosphatidylcholine as the sole phospholipid, had effects on 6-oxo-PGF1α accumulation similar to native HDL, it is likely that HDL-induced Cox-2 elevation in confluent ECs is sufficient to stimulate the eicosanoid in the absence of exogenous arachidonic acid. Furthermore, although studies by Stuhlmeier et al.65 have shown that arachidonic acid can inhibit the TNFα-induced expression of E-selectin by bovine aortic ECs, provision of this component cannot explain the broad spectrum of antiinflammatory effects of HDL, where both VCAM-1 and ICAM-1 are also inhibited.34,48 As recent work by Vinals et al.66 has shown that HDL also induce Cox-2 in vascular smooth muscle cells, it will be interesting to see whether the effect is also enhanced by IL-1β and TNFα.

The mechanism whereby HDL synergizes with cytokines to induce Cox-2 remains to be clarified. It has been shown that EC incubation with HDL can result in the production of lysophosphatidylcholine (LPC), and as exogenous addition of LPC to EC has been demonstrated to elevate not only Cox-2, but also ET-1, another vasoactive agent known to be elevated.

Figure 7. HDLs synergize with the cytokines TNFα and IL-1β to elevate the level of Cox-2 expression in ECs. Western blot analysis of whole cell lysates (10 μg/lane) prepared from confluent ECs preincubated with HDL (1 mg/mL apoAI) alone for 8 hours or preincubated with HDL for 4 hours and then treated with either TNFα (1 ng/mL) or IL-1β (1 ng/mL) and HDL for a further 4 hours. The lanes have been probed with antibody against Cox-2 (Biogenics Ltd). Results are representative of 3 experiments, using HDL from 3 separate donors.

Figure 8. HDL stimulates the accumulation of 6-oxo-PGF1α. After preincubation of confluent ECs with HDL (1 mg/mL apoAI) for 16 hours, IL-1β (1 ng/mL) or TNFα (1 ng/mL) was added for a period of 16 hours after which the supernatants were assayed for levels of 6-oxo-PGF1α. Levels in supernatants from cells incubated with HDL alone (HDL) and cells left untreated (NIL) are shown. The data are representative of 3 separate experiments, using HDL from 3 separate donors, and show mean and SD values. Differences between means were evaluated by using Student’s unpaired t test. *P, not significant; **P<0.001.
HDLs Modulate Expression of E-Selectin and Cox-2

by HDL,\textsuperscript{67} it is tempting to consider that the synergy we have seen is secondary to the elevation in LPC. However, elevation of LPC cannot explain the full actions of HDL, as LPC has been shown to elevate, rather than inhibit, the expression of adhesion molecules that we and others have shown to be inhibited by HDL.\textsuperscript{33,48} In addition, it is possible that HDL may be able to prolong messenger RNA stability of Cox-2, as LPC has been suggested for IL-1 induction of the enzyme.\textsuperscript{68} It is also possible that HDL may mediate its effects transcriptionally. Analyses of the transcriptional requirements of the Cox-2 promoter have been conducted in bovine aortic EC with lipopolysaccharide or 12-O-tetradecanoylphorbol 13-acetate (TPA),\textsuperscript{69} in murine osteoblasts with TNFα,\textsuperscript{70} in rheumatoid synovial fibroblasts by using IL1β,\textsuperscript{71} and in A549 by using IL-1β,\textsuperscript{72} and all provide evidence that NF-κB is required for induced expression of the enzyme. Although our data suggest that HDL does not affect NF-kB translocation or transactivation in ECs, mutational analysis of the effect of HDL on cytokine-induced activation of the Cox-2 promoter may provide important clues as to the mechanism of the synergy between HDL and cytokine.

Although at face value it might seem paradoxical that HDLs inhibit expression of 1 cytokine-induced response while synergistically increasing another, it may be that the differential effects are functionally complementary. Thus, prostacyclin (PGI\textsubscript{2}) has been shown to be a potent inhibitor of leukocyte activation and adhesion, platelet aggregation, and vascular smooth muscle cell contraction, migration, and growth, and it is also shown to inhibit cholesterol ester accumulation in vascular cells.\textsuperscript{73,74} The ability of HDL to enhance TNFα- or IL-1β-mediated prostacyclin production would therefore be expected to act in conjunction with the inhibition of E-selectin, VCAM-1, and ICAM-1 expression to suppress leukocyte–EC interactions and thereby to protect against the progression of atherosclerosis and its thrombotic complications.

Acknowledgments

This work was supported through funding from the British Heart Foundation. G.W.C. is supported by an Intermediate Research Fellowship (FS95/039). D.O.H. is supported by a Professorial Award. The authors wish to thank Dr P. Lerch, Swiss Red Cross, for providing reconstituted discoidal HDL.

References


59. Cockerill et al April 1999


High-Density Lipoproteins Differentially Modulate Cytokine-Induced Expression of E-Selectin and Cyclooxygenase-2
Gillian W. Cockerill, Jeremy Saklatvala, Simon H. Ridley, Helen Yarwood, Norman E. Miller, Barbaros Oral, Saro Nithyanathan, Graham Taylor and Dorian O. Haskard

doi: 10.1161/01.ATV.19.4.910

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/19/4/910

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/