Macrophage lipoprotein lipase expression is increased in patients with heterozygous familial hypercholesterolemia

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Abstract **FH is associated with accelerated atherosclerosis. Based on the crucial role of macrophage LPL in atherogenesis, we determined in the present study macrophage LPL expression in patients with FH. Monocytes isolated from 13 FH patients and 13 control subjects were differentiated into macrophages by culturing the cells for 9 days in 20% autologous or heterologous serum. Macrophages of patients with FH cultured in their own sera showed a significant increase in LPL mRNA levels, extracellular LPL mass, and activity compared with macrophages of control subjects. Although these alterations positively correlated with the levels of serum platelet-derived growth factor-BB (PDGF-BB) in FH subjects, increased LPL secretion by cultured FH macrophages was reduced neither by immunoneutralizing FH serum with an anti-PDGF-BB antibody, nor by culturing these cells in sera from control subjects. With the exception of LPL, levels of other cytokines and 8-isoprostane were not increased in the supernatants of macrophages of FH patients. Serum from FH patients also enhances the levels of LPL secreted by macrophages from control subjects. Immunoneutralization of FH serum with an anti-PDGF-BB antibody totally reversed this alteration. Overall, this study demonstrates that macrophages from FH subjects overproduce LPL and that PDGF present in the serum from FH patients stimulates LPL secretion by control macrophages. These findings suggest that macrophage LPL induction in patients with FH might be related to the increased atherogenesis observed in these subjects.**—Beauchamp, M-C., É. Letendre, and G. Renier. **Macrophage lipoprotein lipase expression is increased in patients with heterozygous familial hypercholesterolemia.** ***J. Lipid Res.*** **2002.** 43: **215–222.**

Supplementary key words atherosclerosis • platelet-derived growth factor • cytokines

Atherosclerosis is an inflammatory lesion, the initiation and progression of which is driven and regulated by immune cells, namely monocytes/macrophages and T lymphocytes (1). Among the earliest events in atherogenesis is the binding of monocytes to endothelium and their migration into the vessel wall (2). Monocyte-derived macrophages present in the arterial wall release various proinflammatory molecules. LPL, a key enzyme in lipid metabolism, is secreted by macrophages in the subendothelial space (3). Evidence has been provided that LPL produced by macrophages in the vessel wall exerts proatherogenic effects. Indeed, it has been demonstrated that LPL content in the arterial wall increases with the progression of the lesion (4), and that a positive correlation exists between its accumulation in the vessel wall and that of cholesterol ester (5). Furthermore, it has been shown that macrophage LPL expression is positively associated with atherosclerotic susceptibility in inbred mice (6), and that this enzyme induces foam cell formation and atherosclerosis in vivo (7, 8). The atherogenic effects of LPL have been mainly attributed to its ability to favor lipid accumulation within macrophages present in the atherosclerotic lesion (9–11). Recently, we and others have proposed new mechanisms through which LPL may promote the development of atherosclerosis, including facilitation of monocyte adhesion to endothelial cells (12–14), stimulation of tumor necrosis factor alpha (TNFα) secretion (15, 16), and induction of vascular smooth muscle cell proliferation (17).

Hypercholesterolemia is a causal factor of atherosclerosis and coronary heart disease. Patients with familial hypercholesterolemia (FH) are at increased risk of premature atherosclerosis (18). Evidence has recently been provided that plasma post-heparin LPL mass and activity are increased in homozygous FH patients compared with the general population, and that these biological parameters correlate with the extent of calcific atherosclerosis in these subjects (19). Despite the key role of macrophage LPL in atherogenesis, the regulation of macrophage LPL in patients with FH has not been studied. The aim of the present study was to characterize macrophage LPL expression in patients with FH.

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HDL-C, high density lipoprotein cholesterol; IL-1β, interleukin-1 beta; IL-6, interleukin-6; LDL-C, low density lipoprotein cholesterol; PDGF, platelet-derived growth factor; TNFα, tumor necrosis factor alpha; 8-*epi* $PGF_{2\alpha}$, 8-isoprostane.

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MATERIALS AND METHODS

Patients

The study group comprised 13 patients with heterozygous FH and 13 healthy control subjects. Patients, five males and eight females, were selected at the Lipid Clinic of the CHUM. These patients were originally diagnosed as having heterozygous FH based on well-defined clinical and biochemical criteria, namely LDL-cholesterol (LDL-C) >95th percentile, presence of tendinous xanthomas, and a familial history of hypercholesterolemia with autosomal mode of transmission. Presence of tendinous xanthomas was demonstrated in eight of the 13 patients (62%). Additional criteria such as increase in LDL-C in the presence of tendinous xanthomas in a first-degree relative or a LDL-C >95th percentile in a child <10 years of age were considered in the absence of tendinous xanthomas. Secondary causes of hypercholesterolemia were excluded by biochemical testing and physical examination. The duration of FH in the study population varied from 2 to 18 years (mean: 10.0 ± 1.4 years). All patients were treated with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. One patient also received bile acid-binding resins (cholestyramine 4 g/day and niacine 500 mg/day). None of the patients received fibrates. Four patients had proven cardiovascular disease and two had arterial hypertension. Four patients were hypothyroid and were treated with leuthyroxine (0.025 to 0.100 mg/day). Two patients received antioxidants (vitamin E 200 IU/day and a multivitamin supplement), and two others were treated by anti-inflammatory drugs. Only one patient was a smoker. Clinical and biochemical characteristics of the study population are shown in **Table 1**.

Protocol

The protocol was approved by the Notre-Dame Hospital Ethics Committee, and informed written consent was obtained from all subjects. Blood samples for lipid and lipoprotein analysis were drawn in the morning after an overnight fast. To assess the role of peripheral factors in macrophage LPL regulation, plasma from control subjects and FH patients was collected from the heparinized blood used for monocyte isolation. Control subjects matched with patients for sex and age were recruited from the hospital staff and relatives. Subjects with infectious or inflammatory conditions were excluded from the study.

Reagents

Fetal calf serum (FCS) and heparin were purchased from Hyclone Laboratories (Logan, UT) and Sigma (St. Louis, MO), respectively. RPMI 1640 medium and Trizol reagent were obtained from Gibco BRL (Burlington, Canada). Anti-platelet derived growth factor-BB (PDGF-BB) and IgG_1 neutralizing antibodies were purchased from R & D Systems (Minneapolis, MN).

TABLE 1. Clinical characteristics of the patients with FH (n = 13)

	FH Patients	Control Subjects
Age (years)	49.3 ± 4.8	38.3 ± 4.0
Triglycerides (mmol/l)	1.55 ± 0.26	1.04 ± 0.13
Cholesterol (mmol/l)		
Total	7.28 ± 0.74[a]	4.64 ± 0.21
LDL	5.46 ± 0.70[a]	2.74 ± 0.09
HDL	1.11 ± 0.08	1.42 ± 0.17
Serum LPL mass (ng/ml)	≤20	≤20
Serum LPL activity (pmol/ml)	3.5 ± 0.3	2.9 ± 0.3

[a] $P < 0.05$.

Measurements of plasma lipids and lipoproteins

All biochemical analyses were performed on a Beckman LX-20 multianalyzer (Beckman, California). Glucose, creatinine, and thyroid stimulating hormone levels were measured to screen for secondary causes of hypercholesterolemia. Lipoprotein fractions were determined on plasma samples obtained after a 14 h fasting period. Plasma total cholesterol concentrations were measured using a cholesterol esterase technique with dosage of the hydrolysis products (20). Levels of HDL-cholesterol (HDL-C) were determined by a timed end-point with dextran-sulfate precipitation. Values of LDL-C were calculated using the Friedewald equation. Triglyceride levels were assayed using a lipase assay with determination of the hydrolysis products (21).

Measurements of serum and extracellular macrophage LPL mass and activity

Preheparin LPL mass and activity in the serum of each subject under study, and LPL immunoreactive mass and activity secreted by macrophages in the supernatants were determined using the Markit-F LPL kit, which measures both active and inactive LPL (Dainippon Pharmaceutical, Osaka, Japan) (22), and the Confluolip kit (Progen, Heidelberg, Germany) (23), respectively. When the secretion media were assayed for LPL, 0.5 U/ml heparin was added to the medium 1 h before the end of the incubation period. For LPL mass values falling below the level of detection, the minimum limit of detection, 20 ng/ml, was used for calculation. Levels of macrophage LPL mass and activity were normalized to levels of total cell proteins.

Human macrophages

Fresh heparinized blood (100 ml) was obtained from patients and healthy donors in the morning. Human monocytes were isolated as previously described (24). Briefly, peripheral blood mononuclear cells were isolated by density centrifugation using Ficoll and allowed to aggregate in the presence of FCS. Enriched monocyte preparations were further depleted in T and NK cells by rosetting with *S*-(2-aminoethyl) isothiouronium bromide-treated sheep red blood cells. Highly purified monocytes (85–90%) were obtained by density centrifugation, as assessed by fluorescence activated cell-sorter (FACS) analysis. Lack of monocyte activation by the isolation procedure was confirmed by determination of the levels of TNFα released by the freshly isolated cells. Differentiation of monocytes into monocyte-derived macrophages was achieved by culturing freshly isolated monocytes in RPMI 1640 medium supplemented with 1% (v/v) penicillin-streptomycin and 20% (v/v) autologous or heterologous serum. The cells were incubated for 8 days at 37°C in a humidified 5% CO_2, 95% air atmosphere. The culture medium was changed at days 4 and 8. Levels of LPL mRNA, mass, and activity were measured 24 h after the last medium change. For experiments aimed at immunoneutralizing FH sera, sera were incubated for 1 h at 37°C with 50 ng/ml PDGF-BB or control IgG_1 isotype antibody, then added to the culture medium of the cells. Fresh immunoneutralized serum was added at days 4 and 8 of culture.

Analysis of LPL mRNA expression

Expression of the LPL gene in human macrophages was performed by PCR. Total RNA for use in the PCR reaction was extracted from human macrophages by an improvement of the acid-phenol technique of Chomczynski and Sacchi (25). Briefly, cells were lysed with Trizol reagent and chloroform was added to the solution. After centrifugation, the RNA present in the aqueous phase was precipitated and resuspended in diethyl pyrocarbonate water. cDNA was synthesized from RNA by incubating 1 μg of total cellular RNA with 0.1 μg oligodT (Pharmacia, Piscat-

away, NJ) for 5 min at 98°C, then by incubating the mixture with reverse transcription buffer for 60 min at 37°C. The cDNA obtained was amplified by using 0.8 μmol/l of two synthetic primers specific for human LPL (5′-GAGATTTCTCTGTATGGCACC-3′) (5′-CTGCAAATGAGACACTTTCTC-3′) and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (5′-CCCTTCATT GACCTCAACTACATGG-3′) (5′-AGTCTTCTGGGTGGCAGTG ATGG-3′) used as internal standard in the PCR reaction mixture. A 277-base pair human LPL cDNA fragment and a 456-base pair human GAPDH cDNA fragment were amplified enzymatically by 22 repeated cycles at 95°C for 60 s, 60°C for 40 s, and 72°C for 90 s. An aliquot of each reaction mixture was then subjected to electrophoresis on a 1% agarose gel containing ethidium bromide. The intensity of the bands was measured by an image analysis scanning system (Alpha Imager 2000; Packard Instruments, Meriden, CT).

Determination of TNFα, interleukin-1β, interleukin-6, and PDGF-BB in the culture media of macrophages

The amounts of macrophage TNFα, interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and PDGF-BB and the concentrations of platelet-poor plasma PDGF-BB were determined by a double-sandwich ELISA (R & D Systems, Minneapolis, MN). Levels of macrophage cytokines were normalized to the levels of total cell proteins. The detection limit of TNFα, IL-1β, IL-6, and PDGF-BB are 4.4 pg/ml, 0.1 pg/ml, 0.094 pg/ml, and 15 pg/ml, respectively. For IL-1β values falling below the level of detection, the lowest value of the standard curve was used for calculation. The intra and interassay coefficients of variation of all these assays are less than 10%.

Determination of 8-isoprostane levels in the supernatants of macrophages

Free 8-isoprostane (8-*epi* $PGF_{2\alpha}$) levels were determined in the culture medium by enzyme immunoassay (Cayman Chemical, Ann Arbor, MI) (26). The detection limit of 8-*epi* $PGF_{2\alpha}$ with this assay is 5 pg/ml. The intra and interassay coefficients of variation of this assay are less than 10%.

Determination of total protein concentration

Total protein content was estimated according to the Bradford method (27) using a colorimetric assay (Bio-Rad, Mississauga, Canada).

Statistical analysis

All values are expressed as the mean ± SEM. Data were analyzed using the non-parametric Mann-Whitney test. The relationship between individual variables was determined by linear correlation analysis. A $P < 0.05$ was considered statistically significant.

RESULTS

Macrophage LPL mRNA levels, immunoreactive mass, and activity in control subjects and FH patients

Macrophages of FH patients cultured in their own sera demonstrated a significant increase in LPL mRNA levels compared with those isolated from control subjects (**Fig. 1A**). Under these experimental conditions, no modulation of the mRNA expression of GAPDH was observed (Fig. 1B). LPL mRNA levels normalized to the levels of GAPDH mRNA are presented in Fig. 1C. [LPL mRNA levels (% over control values): controls: 99.8 ± 1.0; FH patients: 135.0 ± 8.9, (P = 0.007)]. Macrophages of FH patients cultured in their own sera also secreted significantly higher LPL mass (Fig. 1D) and activity (Fig. 1E) than macrophages isolated from control subjects [LPL mass (ng/mg cell proteins/ml): controls: 65.1 ± 13.6; FH patients: 271.2 ± 60.0, P = 0.005); (LPL activity (pmol/mg cell proteins/

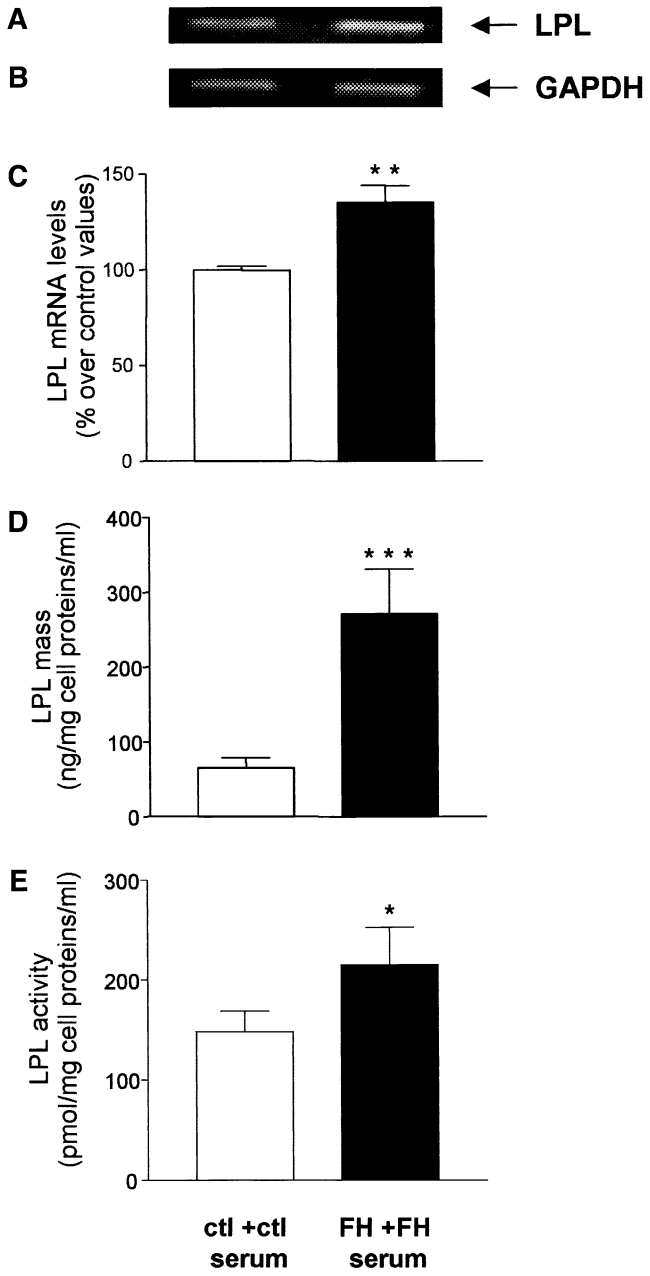

Fig. 1. LPL mRNA levels, immunoreactive mass, and activity in macrophages of control subjects (ctl) and patients with FH. Monocytes isolated from control subjects or patients with FH were cultured for 8 days in RPMI medium containing 20% of their own sera. Twenty-four hours after the last medium change, cells were lysed and LPL (A) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (B) mRNA expression was analyzed by reverse transcription-PCR. C: LPL mRNA levels normalized to the levels of GAPDH levels. LPL immunoreactive mass (D) and activity (E) were determined in the cell supernatants. Results represent the mean ± SEM of data obtained from 13 control subjects and 13 patients with FH. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus controls.

ml): controls: 148.4 ± 20.5; FH patients: 215.0 ± 37.5, $P = 0.036$].

Pre-heparin serum LPL mass and activity in control subjects and FH patients

To determine whether increased levels of LPL mass and activity in the sera of FH patients might be responsible for the induction of macrophage LPL in FH patients, the levels of serum LPL mass and activity were determined in control subjects and FH patients. Preheparin serum LPL mass and activity values did not significantly differ from those measured in the control subjects (Table 1).

Correlation between platelet-poor plasma PDGF-BB levels and macrophage gene expression, mass, and activity in patients with FH

Because serum PDGF levels are increased in patients with FH (28), and that PDGF enhances macrophage LPL expression in vitro (29), we next determined the levels of plasma PDGF-BB in seven control subjects and seven FH patients and evaluated whether a correlation exists between this parameter and macrophage LPL gene expression and secretion. Plasma PDGF-BB concentrations were significantly higher in patients with FH than in control subjects [PDGF-BB (pg/ml): controls: 129.4 ± 36.9; FH patients: 292.1 ± 44.1, $P = 0.013$]. In contrast, PDGF-BB levels in the supernatants harvested from macrophages did not differ between the control and FH groups [PDGF-BB (pg/mg cell proteins/ml) controls: 79.81 ± 21.22: FH patients: 84.29 ± 22.77]. A positive correlation between plasma PDGF-BB levels and macrophage LPL mRNA levels ($r = 0.680$, $P = 0.092$) (**Fig. 2A**), mass ($r = 0.827$, $P = 0.02$) (Fig. 2B), and activity ($r = 0.824$, $P = 0.02$) (Fig. 2C) was found.

Relationship between macrophage LPL mass/activity and lipid profile

No significant correlation was found between levels of macrophage LPL mass or activity and parameters of lipid profile in the study population. Coefficients of correlation between macrophage LPL mass and total cholesterol or LDL cholesterol were $r = 0.559$ ($P = 0.093$) and $r = 0.559$ ($P = 0.093$), respectively. Coefficients of correlation between macrophage LPL activity and total cholesterol or LDL cholesterol were $r = 0.463$ ($P = 0.111$) and $r = 0.423$ ($P = 0.150$), respectively.

Extracellular LPL immunoreactive mass and activity in macrophages of control subjects and FH patients cultured in heterologous serum

Culture of macrophages of control subjects in sera from FH patients significantly induced LPL mass and activity: [LPL mass (ng/mg cell proteins/ml): control cells cultured in control serum: 65.1 ± 13.6; control cells cultured in FH serum: 102.3 ± 6.0, $P < 0.001$], (**Fig. 3A**); LPL activity (pmol/mg cell proteins/ml): control cells cultured in control serum: 148.4 ± 20.5; control cells cultured in FH serum: 255.4 ± 20.3, $P < 0.001$), (Fig. 3B). In contrast, differentiation of macrophages from FH patients in the sera from control subjects did not significantly reduce the levels of LPL mass (Fig. 3A) and activity (Fig. 3B) secreted by these cells. [LPL mass (ng/mg cell proteins/ml): FH cells cultured in FH serum: 271.2 ± 60.0; FH cells cultured in control serum: 220.5 ± 22.3, $P > 0.05$; LPL activity (pmol/mg cell proteins/ml): FH cells cultured in FH serum: 215.0 ± 37.5; FH cells cultured in control serum: 226.5 ± 34.7, $P < 0.05$.]

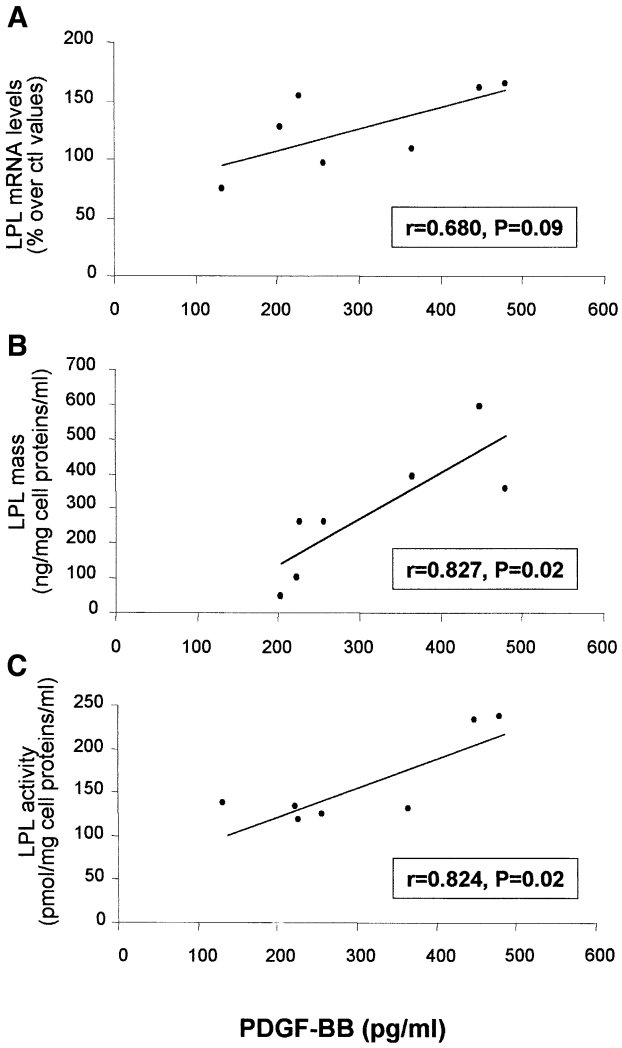

Fig. 2. Scatterplots showing the correlations between platelet-poor plasma platelet-derived growth factor-BB (PDGF-BB) levels in FH patients (n = 7) and LPL mRNA levels (A), immunoreactive mass (B) and activity (C) from macrophages of FH patients cultured in their own sera.

Effect of PDGF-BB immunoneutralization of FH sera on macrophage LPL secretion in control subjects and FH patients

To evaluate the role of PDGF-BB in the increased LPL secretion that we documented in macrophages of control subjects cultured in sera from FH patients, control macrophages were differentiated for 8 days in PDGF-BB- or IgG_1-immunoneutralized sera from eight patients with FH. Twenty-four hours after the last medium change, the amount of LPL mass and activity secreted by these cells

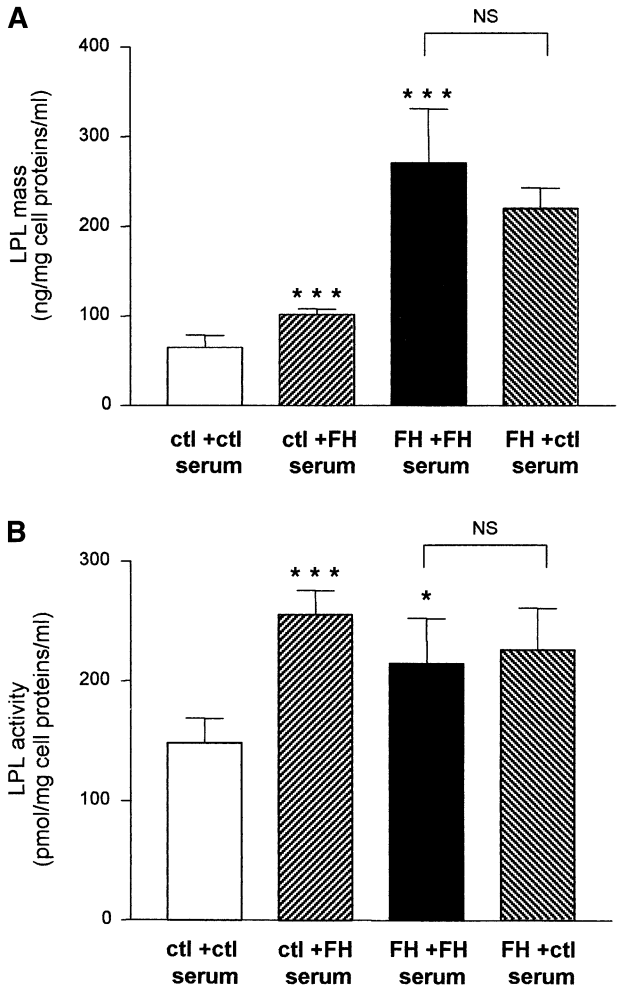

Fig. 3. LPL immunoreactive mass and activity secreted by macrophages of control subjects (ctl) and patients with FH. Monocytes isolated from control subjects (n = 13) and patients with FH (n = 13) were cultured for 8 days in RPMI medium containing 20% of their own sera. In some experiments, monocytes of control subjects were differentiated in sera of FH patients (n = 7), and monocytes of FH patients were differentiated in sera of control subjects (n = 7). Twenty-four hours after the last medium change, LPL immunoreactive mass (A) and activity (B) were determined in the cell supernatants. * $P < 0.05$, *** $P < 0.001$ versus controls.

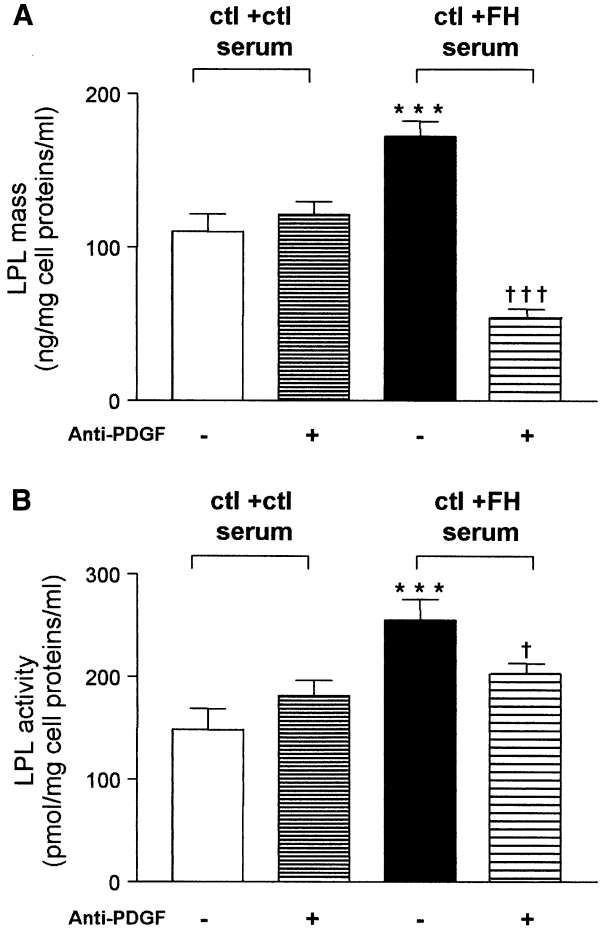

Fig. 4. LPL immunoreactive mass (A) and activity (B) secreted by control macrophages cultured in PDGF-BB- (50 ng/ml) immunoneutralized FH sera. Monocytes isolated from control subjects were cultured for 8 days in RPMI medium containing 20% control sera, PDGF-BB-immunoneutralized control sera, FH sera or PDGF-BB-immunoneutralized FH sera. Twenty-four hours after the last medium change, LPL immunoreactive mass (A) and activity (B) were determined in the medium. *** $P < 0.001$ versus ctl macrophages + ctl serum; † $P < 0.05$, ††† $P < 0.001$ versus ctl macrophages + FH serum.

were measured. Control macrophages cultured in PDGF-BB-immunoneutralized FH sera showed a dramatic decrease in LPL mass (**Fig. 4A**) and activity (Fig. 4B) as compared with those cultured in non-immunoneutralized FH sera. In contrast, immunoneutralization of FH sera with IgG_1 did not affect the levels of LPL mass and activity secreted by these cells [LPL mass (ng/mg cell proteins/ml): control cells cultured in FH serum: 102.3 ± 6.0; control cells cultured in IgG_1-immunoneutralized FH serum: 124 ± 7.0, $P > 0.05$; LPL activity (pmol/mg cell proteins/ml): controls cells cultured in FH serum: 255.4 ± 20.3; control cells cultured in IgG_1-immunoneutralized FH serum: 254.2 ± 28.7, $P > 0.05$].

The effect of PDGF-BB immunoneutralization of FH sera on LPL secretion by FH macrophages was also evaluated. FH macrophages cultured in PDGF-BB-immunoneutralized FH sera showed similar levels of LPL secretion as compared with FH cells cultured in non-immunoneutralized or in IgG-immunoneutralized FH sera. [LPL mass (ng/mg cell proteins/ml): FH cells cultured in FH serum: 271.2 ± 60.0; FH cells cultured in IgG_1-immunoneutralized FH serum: 215.7 ± 16.9; FH cells cultured in PDGF-BB-immunoneutralized FH serum: 251.5 ± 34.2, $P > 0.05$).] [LPL activity (pmol/mg cell proteins/ml): FH cells cultured in FH serum: 215.0 ± 37.5; FH cells cultured in IgG-immunoneutralized FH serum: 196.0 ± 18.9; FH cells cultured in PDGF-BB-immunoneutralized FH serum: 207.6 ± 26.8, $P > 0.05$.]

Levels of TNFα, IL-1β, IL-6, and 8-*epi* $PGF_{2\alpha}$ in the culture media of macrophages of control subjects and FH patients

To finally evaluate whether increased expression of macrophage LPL of FH subjects may reflect a generalized

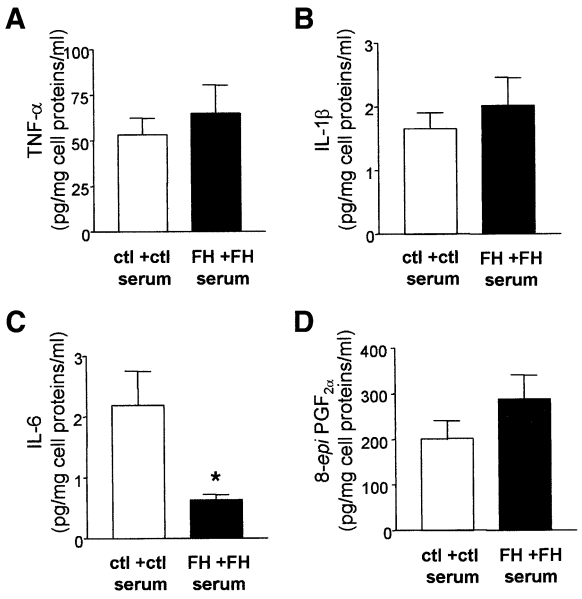

Fig. 5. Levels of tumor necrosis factor alpha (TNFα) (A), interleukin-1 beta (IL-1β) (B), interleukin-6 (IL-6) (C), and 8-isoprostane (8-*epi* $PGF_{2\alpha}$) (D) in the supernatants of macrophages of control subjects (ctl) or patients with FH. Monocytes isolated from control subjects or patients with FH were cultured for 8 days in RPMI medium containing 20% of their own sera. Twenty-four hours after the last medium change, the levels of TNFα, IL-1β, IL-6, and 8-*epi* PGF2α were determined in the medium. Results represent the mean ± SEM of data obtained from 13 control subjects and 13 patients with FH. * $P < 0.05$ versus controls.

activation state of these cells, we next measured basal TNFα, IL-1β, IL-6, and 8-*epi* $PGF_{2\alpha}$ levels in the supernatants of macrophages of control subjects and FH patients. Macrophage TNFα and IL-1β production was similar in the FH and control groups [TNFα (pg/mg cell proteins/ml) controls: 53.3 ± 9.1; FH patients: 65.0 ± 15.5, $P = 0.515$; IL-1β (pg/mg cell proteins/ml) controls: 1.65 ± 0.26; FH patients: 2.013 ± 0.44, $P = 0.418$] (**Fig. 5A** and **B**). In contrast, a significant decrease in macrophage IL-6 secretion was observed in the FH group [IL-6 (pg/mg cell proteins/ml) controls: 2.19 ± 0.56; FH patients: 0.63 ± 0.09, $P = 0.023$] (Fig. 5C). There was a tendency toward increase in the 8-*epi* $PGF_{2\alpha}$ levels in the FH patients, though not statistically significant [8-*epi* $PGF_{2\alpha}$ (pg/mg cell proteins/ml) controls: 199.1 ± 42.1; FH patients: 287.4 ± 52.9, $P = 0.207$] (Fig. 5D).

DISCUSSION

Evidence has been provided that secretion of LPL by macrophages in the arterial wall promotes the development of atherosclerosis in vivo (7, 8). The present study demonstrates that macrophages isolated from patients with FH express higher levels of LPL mRNA and secrete larger amounts of LPL than macrophages isolated from control normolipidemic subjects. The parallel increase of LPL mRNA levels and LPL immunoreactive mass/activity observed in our study indicates a pre-translational control of macrophage LPL expression in FH patients. The mechanism(s) underlying the induction of macrophage LPL mRNA expression in FH patients may involve both transcriptional and/or post-transcriptional events. Although the limited amounts of biological material extracted from human cells did not allow us to perform run-on experiments or to measure LPL mRNA stability, upregulation of macrophage LPL gene expression in FH may theoretically occur at the transcriptional level as a result of increased serum PDGF-BB and/or cholesterol levels. Indeed, evidence has been provided that PDGF-BB upregulates human macrophage LPL mRNA levels (29), and that LPL expression is subject to regulation by cellular sterol levels through interaction of sterol regulatory element binding proteins (SREBP), with a sterol regulatory element spanning from −90 to −81 in the LPL promoter (30, 31).

Although the mechanisms responsible for macrophage LPL induction in FH remain uncertain, our finding that culture of FH macrophages in control sera does not significantly reduce this alteration indicates that peripheral factors are not key determinants of macrophage LPL overproduction in FH. Specifically, our results, which show that neutralization of PDGF in FH sera does not reduce the levels of LPL secreted by FH macrophages, clearly demonstrate that, despite the positive association between serum PDGF-BB levels and macrophage LPL expression in FH patients, PGDF-BB is not the cause of macrophage LPL overproduction in patients with FH. LPL induction associated with FH may rather reflect some genetically controlled differences in LPL expression in the FH macrophage population. This possibility is supported by our previous observation that high macrophage LPL expression is associated with susceptibility to atherosclerosis in inbred mice (6), and that alteration of intracellular cholesterol metabolism regulates the steady state levels of LPL mRNA (31). Alternatively, because inflammatory macrophages produce large amounts of LPL, macrophage LPL upregulation in subjects with FH may reflect a general pro-inflammatory monocyte/macrophage cytokine profile in hypercholesterolemic patients. It has been previously suggested that FH could be associated with some alterations of monocyte/macrophage function, such as enhanced TNFα production in response to lipopolysaccharide (32, 33). To assess the state of activation of macrophages of patients with FH, we measured the ability of these cells to spontaneously release cytokines and generate 8-*epi* $PGF_{2\alpha}$ in the supernatants. Our results, which demonstrate that FH macrophages do not produce higher amounts of cytokines and 8-*epi* $PGF_{2\alpha}$ than control cells, clearly indicate that these cells are not inflammatory macrophages and that inflammation may therefore not account for the increase in macrophage LPL expression described here.

Along with the observation that macrophages from FH patients overproduce LPL, we found that serum from FH patients increases LPL levels in macrophages from control subjects. Determination of the levels of LPL mass released by control macrophages exposed to FH sera demonstrates that the amounts of LPL mass secreted by

these cells were much lower than those produced by macrophages from FH subjects. These data clearly indicate that peripheral factors present in the sera from FH patients stimulate LPL secretion by control macrophages, and further stress the marginal role of these factors in the dysregulation of macrophage LPL secretion in patients with FH. Because increase in post-heparin serum LPL mass and activity levels has been reported in patients with homozygous FH (19), accumulation of LPL in the sera of FH patients may account for the induction of macrophage LPL in patients with FH. Our results, which demonstrate that pre-heparin serum LPL mass and activity do not differ in the control and FH groups, do not argue for a role of this factor in the dysregulation of macrophage LPL that we documented herein. Other LPL-stimulatory factors that may accumulate in the serum of FH subjects include triglycerides (34), total or LDL cholesterol (35), and PDGF (28). Because all the patients recruited in our study were normotriglyceridemic, hypertriglyceridemia does not seem to account for macrophage LPL alteration in patients with FH. Alternatively, macrophage LPL may be induced by hypercholesterolemia. Although we did not establish a statistically significant correlation between macrophage LPL and plasma cholesterol levels in patients with FH, the currently documented correlation coefficients of 0.56 may be suggestive given the small sample size in the present study. To the best of our knowledge, only one study has so far examined the in vitro effect of cholesterol on human macrophage LPL regulation. Although this study reported a slight increase in LPL activity and mRNA levels in macrophages exposed to native LDL, this effect did not achieve statistical significance (35). From these data, it appears that definitive conclusions about the role of cholesterol in the control of macrophage LPL require further study. Because PDGF is increased in the serum of heterozygous FH patients (28) and stimulates human macrophage LPL expression in vitro (29), this factor may be involved in the upregulation of macrophage LPL in control cells differentiated in FH sera. Our finding that circulating PDGF-BB levels are increased in FH patients, and that PDGF-BB immunoneutralization of FH sera markedly decreases LPL secretion by control macrophages exposed to sera from FH patients, clearly supports a role of this factor as a macrophage LPL stimulatory molecule.

Dugi et al. have recently demonstrated that a positive correlation exists between post-heparin plasma LPL and the extent of calcific atherosclerotic lesions in these subjects (19). These authors have proposed that elevation of plasma LPL could reflect the massive production of LPL by macrophages in the atherosclerotic arterial vessel and may therefore represent the consequence of the developing atherosclerosis in these patients. In accordance with Dugi's hypothesis, the present study demonstrates that macrophages isolated from patients with heterozygous FH overproduce LPL. This observation suggests that FH may tend to increase the in vivo production of LPL in the arterial wall and that high LPL secretion may contribute to the development and progression of the atherogenic process taking place in the vessel wall of subjects with FH. Given the small number of patients recruited in this study and the well-documented heterogeneity of the FH population, prospective randomized clinical studies in a large number of newly diagnosed FH patients, before and after therapeutic interventions, are imperative. These studies may provide further insight into the role of macrophage LPL in the accelerated atherosclerosis associated with FH.

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