Vol. 126, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 16, 1985 Pages 526-531

CELLS OF A HUMAN MONOCYTIC LEUKEMIA CELL LINE (THP-1) SYNTHESIZE AND SECRETE APOLIPOPROTEIN E AND LIPOPROTEIN LIPASE

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Received November 28, 1984

SUMMARY: A human cell line established from a patient of an acute monocytic leukemia (THP-1) retained an ability to synthesize and secrete plasma apolipoprotein E like protein. The protein was identified with monospecific antibody raised against human plasma apolipoprotein E. The cells also secreted lipoprotein lipase (EC 3.1.1.34). The enzyme was characterized as lipoprotein lipase on the basis of the requirement of apolipoprotein C-II as an activator and the inhibition of its activity by sodium chloride. The secretion of both apolipoprotein E and lipoprotein lipase was markedly enhanced in the process of differentiation into macrophage-like cells by the addition of 4β-phorbol 12β-myristate 13α-acetate.

It has been suggested that macrophages play an important role in the development of atherosclerotic lesions (1). Recently, Basu et al. reported that mouse peritoneal and human monocyte-derived macrophages secrete apolipoprotein E (apoE) (2,3). It was also shown that rabbit alveolar macrophages, human monocyte-derived macrophages, and macrophage-like cells of a line J774 secrete lipoprotein lipase activity (4-7). Both apoE and lipoprotein lipase play important roles in plasma lipoprotein metabolism: ApoE recognizes low-density-lipoprotein and apoE receptors (1,8) and lipoprotein lipase is the key enzyme of the hydrolysis of triglyceride in lipoproteins (9).

To investigate the lipoprotein metabolism by macrophages, it is advantageous to utilize a human monocyte cell line that is easy to culture or to obtain in large scale. For these reasons we have studied whether or not apoE

Abbreviations: apolipoprotein C-II; apoC-II, apolipoprotein E; apoE, 4β-phorbol 12β-myristate 13α-acetate; TPA, sodium dodecylsulfate; SDS.

0006-291X/85 $1.50

and lipoprotein lipase are secreted by cultured cells (THP-1), which had been established as a cell line from a patient of an acute monocytic leukemia, having an ability to differentiate into macrophages by 4β-phorbol 12β-myristate 13α-acetate (TPA) (10,11).

This study provided evidence that THP-1 cells secrete apoE and lipoprotein lipase into the culture medium, especially after the cells are differentiated into macrophages.

MATERIALS AND METHODS

Purification of apolipoprotein C-II and E: Apolipoprotein C-II (apoC-II) and apoE were isolated from the plasma lipoprotein fractions of a density less than 1.019, from human subjects with type I and V hyperlipoproteinemias. Apo-C-II was purified on Sephadex G-200 and DEAE Sephadex A-25 columns in the presence of denaturants (12). ApoE was purified from the eluate of the Sephadex G-200 column by using a heparin-Sepharose column in the presence of 8 M urea, and a Sephacryl S-300 column (1.5 x 150 cm) in the presence of 6 M guanidine hydrochloride and 0.1% β-mercaptoethanol.

Raising of Monospecific Antibodies against ApoE: One hundred micrograms of purified apoE was mixed with equal volume of Freund's Complete Adjuvant (Iatron, Tokyo) and injected subcutaneously into rabbits at intervals of two weeks. Its activity and specificity were measured by using immunoblotting analysis (13). Specific antibodies against apoE were enriched from the anti-serum by apoE-conjugated Sepharose 4B. The eluted antibodies were dialyzed against phosphate-buffered saline containing 0.02% sodium azide and then concentrated by a membrane filter (Amicon Model 12) using PM-30 membrane, to a final concentration of 0.5-1.0 A_{280} unit/ml.

Cells: Stock culture of THP-1 was maintained in a humidified incubator (5% CO_2) at 37°C in RPMI 1640 medium supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), and 10% (v/v) fetal calf serum (Armour). For experiments, 5 x 10^5 cells of THP-1 were seeded into 30-mm Petri dishes containing 1 ml of fresh medium containing 10% fetal calf serum and 200 ng/ml of TPA, and cultured for 2-4 days.

Monolayers of human monocytes were prepared by the method of Bøyum (14) using Lymphocyte Separation Medium (Bionetics) from 10 ml of blood. The mononuclear cells were collected from the interphase, washed once with phosphate-buffered saline, and suspended in 3 ml of RPMI 1640 containing 20% fetal calf serum. One ml aliquots of the suspension were transferred into three 30-mm Petri dishes. The cells were incubated in a humidified incubator (5% CO_2) at 37°C. After 1 h, the non-adherent cells were removed. The monolayers were cultured for 7 to 10 days, being supplemented with 1 ml of fresh medium containing 20% fetal calf serum at intervals of 2-3 days.

Protein Synthesis and Secretion by Cultured Cells and Immunoprecipitation of ApoE: The suspended cells and monolayers of THP-1, and monocyte-derived macrophages in 30-mm Petri dish were incubated with 1 ml of methionine-free Eagle's minimal essential medium (Nissui Seiyaku, Tokyo) containing 40 μM of unlabeled L-methionine, 8 μg/ml of insulin (3), 6 μg/ml of kanamycin, and 20-40 μCi/ml of [^{35}S]methionin at 37°C for 24 h. The medium was collected and apoE was isolated by immunoprecipitation with monospecific anti-apoE antibodies as described by Gerace and Blobel (15). Protein A-Sepharose was used as immunoadsorbent. The immunoprecipitate was analyzed by sodium dodecylsulfate(SDS)-polyacrylamide 15% cross-linked gel electrophoresis (16) and subsequent fluorography of DPO-impregnated gels (17). ^{14}C-labeled proteins (13) were used as molecular weight markers.

Assay of Lipoprotein Lipase Activity: The incubation mixture contained 3.3% fatty acid-free bovine serum albumin (Sigma), 0.13 M Tris-HCl (pH 8.2), 67 μg/ml sodium heparin (Sigma), 0.09 M sodium chloride, 3.05 mM tri[1-^{14}C]olein (0.069 μCi/μmol triolein), in the presence or absence of 1 μM apoC-II, and cultured medium containing lipoprotein lipase in a final volume of 0.30 ml. Tri[1-^{14}C]olein was emulsified with Branson Sonifier in the presence of 2.5% gum arabic and used as a substrate. The reaction mixture was incubated at 37°C for 30 min. The fatty acid was extracted by the addition of 3 ml of a chloroform-heptane-methanol extraction mixture as described by Belfrage and Vaughan (18). The radioactivity was determinied with a Beckman LS-3800 liquid scintillation counter.

RESULTS AND DISCUSSION

Synthesis and Secretion of ApoE: Cultured macrophages derived from human blood monocytes secreted apoE. This apoE was more acidic and appeared to be larger in molecular weight than the plasma apoE (Ref. 3, Fig.1 lane 2). Cultured monolayers of THP-1 cells which were differentiated by TPA also secreted apoE. The mobility of this apoE was the same as that of apoE secreted by human blood monocyte-derived macrophages (Fig.1 lane 3). Addition of unlabeled plasma apoE in the immunoprecipitation reaction mixture extinguished the bands of [^{35}S]apoE secreted by THP-1 and blood monocyte (Fig.1 lanes 4 and 5), showing that the apoE-like protein synthesized and secreted by THP-1 was apoE. THP-1 cells remaining as monocytes could proliferate, but did not adhere to the

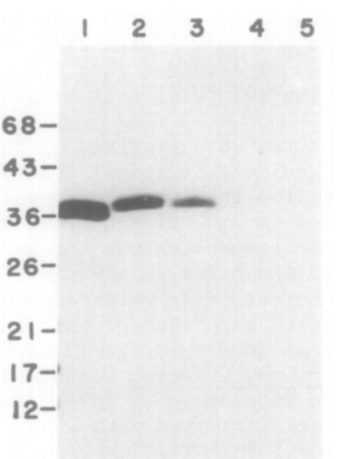


Fig. 1: SDS-polyacrylamide gel electrophoresis and fluorography of immunoprecipitated ^{35}S-labeled apoE secreted by THP-1 (lanes 2 and 4) and human monocytes (lanes 3 and 5). The cultured cells were added with 40 μCi/ml of [^{35}S]-methionine and incubated for 24 h. Aliquots of the medium were immunoprecipitated with anti-apoE antibodies in the absence (lanes 2 and 3) or in the presence of 15 ug of unlabeled plasma apoE (lanes 4 and 5). Lane 1 shows ^{14}C-labeled plasma apoE. M_r (x 10^{-3}) standards are indicated.

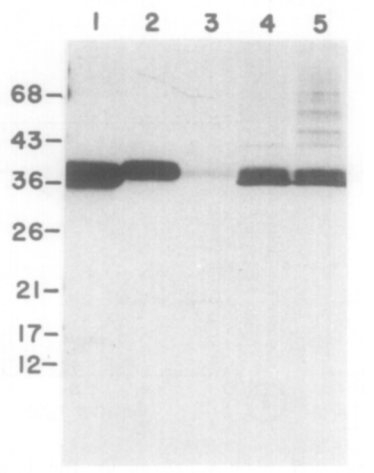
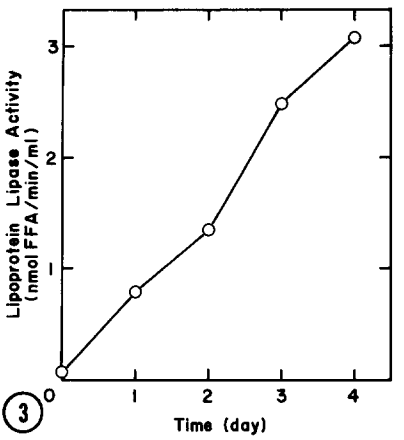


Fig. 2:SDS-polyacrylamide gel electrophoresis and fluorography of immunoprecipitated ^{35}S-labeled apoE synthesized by THP-1 cells in suspension or differentiated into macrophages. The cells were cultured in the presence (lanes 2 and 4) or in the absence (lanes 3 and 5) of TPA. Then the cells were added with 20 μCi/ml of [^{35}S]methionine and were incubated for 24 h. The medium (lanes 2 and 3) and the cells (lanes 4 and 5) were immunoprecipitated with anti-apoE antibodies. Lane 1 shows ^{14}C-labeled plasma apoE. M_r (x 10^{-3}) standards are indicated.

culture dish unless they received TPA. By the addition of TPA, almost all the cells differentiated into macrophages and stopped cell division (11). We investigated whether or not the differentiation of THP-1 into macrophages affected the synthesis or secretion of apoE. As shown in Fig.2, the secretion of the apoE into the medium was markedly increased by the addition of TPA into the culture medium (lanes 2 vs 3). The apoE inside the cells was not so much increased by the differentiation (lanes 4 vs 5).

Secretion of Lipoprotein Lipase: It was also reported that human blood monocyte-derived macrophage secretes lipoprotein lipase activity (4-6). We examined whether or not the THP-1 cells retained the property of secreting lipoprotein lipase. Figure 3 shows the secretion of the apo C-II dependent lipase activity monitered every 24 h in the culture medium of THP-1 cells before and after the addition of TPA. In the absence of TPA, THP-1 cells secreted the lipase activity at a very low level, but the secretion was markedly enhanced following the addition of TPA. The enzyme activity was dependent on apoC-II concentration and was inhibited by sodium chloride (Fig. 4A and B). This indicates that THP-1 cells secreted lipoprotein lipase, and the secretion was



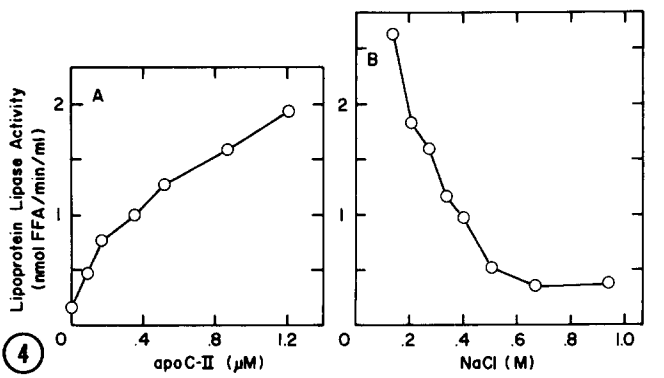


Fig. 3:Lipoprotein lipase activity secreted by THP-1 cells. Lipoprotein lipase activity was monitored every 24 h in the culture medium of THP-1 cells following the addition of 200 ng/ml of TPA. The activity at time 0 shows the lipoprotein lipase activity accumulated in the culture medium during 24 h before the addition of TPA.

Fig. 4:Effects of apoC-II (A) and NaCl (B) on the activity of lipoprotein lipase secreted by THP-1 cells into the culture medium.

markedly stimulated by the differentiation of the cells into macrophages. Lipoprotein lipase activity secreted by THP-1 cells was almost equal to that secreted by human monocyte-derived macrophages (the activity of 3-7 nmol free fatty acid/min/ml was secreted by macrophages prepared from 10 ml of blood).

The established human monocyte cell line THP-1 retained the ability to secrete apoE and lipoprotein lipase. The secretion of these proteins were markedly enhanced in the process of differentiation into macrophages. This cell line could be a useful model in investigation of the lipoprotein metabolism by macrophages.

ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in Aid for Encouragement of the Young Scientists No.59780215 from the Ministry of Education, Science and Culture of Japan.

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