Oncostatin M Up-regulates Low Density Lipoprotein Receptors in HepG2 Cells by a Novel Mechanism*

(Received for publication, May 2, 1991)

Robert I. Grove†, Charles E. Mazzucco, Susan F. Radka, Mohammed Shoyab, and Peter A. Kiener

Oncostatin M is a growth regulatory protein secreted by macrophages and activated T lymphocytes. In a hepatoma cell line (HepG2) the polypeptide very potently increased low density lipoprotein (LDL) uptake with an EC50 of 0.1–0.2 nM. The stimulation of LDL uptake was detectable by 2 h, was maximal by 8 h, and remained elevated through 20 h of oncostatin M incubation. In a similar fashion, oncostatin M also increased the number of cell surface LDL receptors by a mechanism that was inhibited by cycloheximide or the protein kinase C inhibitor H-7. Oncostatin M stimulation of LDL uptake and receptor protein occurred regardless of the state of cholesterol-dependent regulation of HepG2 LDL receptor (i.e. cells incubated in medium containing lipoproteins responded to the same extent as did cells incubated in the absence of lipoproteins). No significant effects were observed on sterol synthesis over 6 h or on DNA synthesis over 24 h.

Oncostatin M induced rapid alterations in HepG2 phospholipid metabolism. Within 5–15 min there was a 20–50% increase in incorporation of 32P into several classes of phospholipids, including the phosphoinositides. Radiolabeled diacylglycerol levels were elevated 20% by 2 min and nearly 50% by 15 min. In addition, the polypeptide induced rapid increases (within 1 min) in phosphorylation of HepG2 proteins on tyrosine residues. Stimulation of both phosphotyrosine and LDL receptor up-regulation by oncostatin M was decreased by the tyrosine kinase inhibitor genistein. We propose that oncostatin M up-regulates HepG2 LDL receptor expression by a mechanism that includes stimulation of a tyrosine kinase followed by generation of phospholipid-related second messengers.

The hepatic low density lipoprotein (LDL) receptor plays a major role in cholesterol homeostasis (1–4). Circulating cholesterol in the form of LDL is removed from plasma by the highly specific LDL receptor and is internalized via receptor-mediated endocytosis. Upon degradation of the LDL particle in the lysosomal compartment, the LDL-derived cholesterol elevates the intracellular free cholesterol concentration. The elevated free cholesterol (or an oxysterol derivative) signals the hepatocyte to decrease transcription of the message of some of the key enzymes in the cholesterol biosynthetic pathway (5), resulting in a decrease in de novo cholesterol synthesis. LDL receptor message and protein are also down-regulated by elevated intracellular cholesterol (5, 6), resulting in decreased ability of the liver to remove additional LDL cholesterol from the plasma (3, 7, 8). A mechanism to up-regulate the LDL receptor independently would therefore be expected to yield an additional decrease in plasma cholesterol levels (4).

Mono nucleolar leucocytes secrete a number of polypeptides that modulate a wide variety of different cellular functions, including proliferation and differentiation (9). Recently, evidence that macrophages may affect cholesterol homeostasis has been emerging. Colony stimulating factors that activate macrophages cause a dramatic decrease in total serum cholesterol in humans (10) and in primates (11). A study in diet-induced hypercholesterolemic rats indicated that injection of a macrophage activator (zymosan) caused a significant decrease in total serum cholesterol (12). More recent evidence has suggested that endotoxin-stimulated macrophage-conditioned media were able to induce a significant increase in uptake of LDL and in LDL receptor number in a human liver cell line.* The conditioned media also caused a decrease in cholesterol synthesis which did not appear to be related to the LDL receptor up-regulation. Further analysis of the conditioned media indicated the majority of the LDL receptor up-regulatory activity was caused by oncostatin M (13).

Originally discovered in conditioned media from a human macrophage cell line, oncostatin M is a novel glycoprotein that has growth regulatory properties. Depending on the cell type, the polypeptide may or may not alter proliferation (14). Oncostatin M has a molecular mass of 28 kDa and binds to high affinity cell surface receptors of approximately 150 kDa as determined by cross-linking studies (15). In the current study, we have characterized the interaction of oncostatin M with HepG2 cells and report on the mechanism by which it up-regulates LDL receptors.

**MATERIALS AND METHODS**

*Cells and Reagents—The hepatoma cell line HepG2 was obtained from ATCC (Bethesda, MD) and cultured in RPMI medium supplemented with 10% fetal bovine serum. Cell culture reagents were obtained from GIBCO except for lipoprotein-deficient serum and the fluorescent Dil-LDL (Biotechnologies Inc., Boston). Genistein was from ICN. Oncostatin M and monoclonal antibodies were from Oncogen (Seattle). The OM1 and OM2 monoclonal antibodies are directed against epitopes on native oncostatin M; OM1 did not neutralize the effects of oncostatin M in a growth inhibition assay whereas OM2 completely abrogated oncostatin M-induced activity.

†To whom correspondence should be sent: Oncogen, Bristol-Myers Squibb PRI, 3005 First Ave., Seattle, WA 98121. Tel.: 206-728-0640; Fax: 206-728-9263.

‡The abbreviations used are: LDL, low density lipoprotein; EGF, epidermal growth factor; BES, 2-[bis(2-hydroxyethyl)amino]ethanesulfonic acid; PDGF, platelet-derived growth factor; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; LFDS, lipoprotein-depleted serum; ELISA, enzyme-linked immunosorbent assay.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 R. I. Grove, C. Mazzucco, P. Kiener, S. F. Radka, M. Shoyab, and G. War. manuscript submitted for publication.

3 S. F. Radka, P. Linsley, and M. Shoyab, manuscript submitted for publication.
Rabbit anti-phosphotyrosine antisera were raised and purified essentially as described by Kamps and Sefton (10) and were a gift from Dr. J. Ledbetter (Oncogen). On occasion, a commercial anti-phosphotyrosine antibody raised against tyrosine phosphorylated v-abl peptide (Pharmingen) was also used. Radiolipids were purchased from Du Pont-New England Nuclear. All other chemicals were reagent grade or better.

**LDL Uptake Assay**—LDL uptake in HepG2 cells cultured in 12-well plates (Costar) at 2 × 10⁵/well was assayed by the fluorescent LDL method (17–19) using a fluorescence microscope and video camera (SIT 66) attached to a computer (Laser 25) with JAVA image analysis software (Jandel Scientific, CA) as reported previously. Phorbol esters were incubated with HepG2 monolayers for 2 h. The monolayers were washed three times to remove free Dil-LDL and fixed with 4% formalin in phosphate-buffered saline. Accumulated fluorescence was measured by quantitating the average intensity of 25–50 cells. Three different fields in each of duplicate or triplicate monolayers were quantitated and averaged. The amount of LDL protein taken up was determined as follows. Cell monolayers (unfixed) were solubilized with 0.1% sodium dodecyl sulfate, transferred into glass tubes, and the lipids (and Dil) were extracted into chloroform using the Bligh-Dyer extraction procedure (20). Fluorescence in the chloroform layer was quantitated with a fluorescence spectrophotometer (Perkin-Elmer) and compared with a standard curve generated by extracting Dil from known quantities of Dil-LDL. Protein was estimated using Coomassie Blue kits (Pierce Chemical Co.).

**LDL Receptor ELISA**—The amount of surface LDL receptor was estimated with an ELISA that employed the LDL receptor antibody C-7 (21). The HepG2 monolayers were fixed with 4% formalin and blocked for 2 h with phosphate-buffered saline (PBS) containing 3% (w/v) bovine serum albumin. The blocking solution was replaced with PBS containing bovine serum albumin (0.5%) and 5 µg/ml C-7 and incubated at 25 °C for 2 h. The monolayers were washed extensively to remove excess antibody with PBS, 0.5% bovine serum albumin, 0.05% Triton X-100 and then incubated for 1 h in PBS containing 20 µg/ml peroxidase-conjugated goat anti-mouse IgG (Cooper Biochemicals). Excess antibody was removed, and peroxidase substrate (O-diphenylenediamine (4 mg/ml) and hydrogen peroxide (0.00012%) in 0.1 M citrate phosphate, pH 5.0) was added to the monolayers. Color development was stopped after approximately 10 min by the addition of HCl (2.5 N, final concentration). Optical density was measured at 490 nm in a microtiter plate reader (Molecular Devices).

**Sterol Synthesis Assay**—Sterol synthesis was estimated from the sterol synthesis method of Mosley et al. (22). Briefly, cell cultures were incubated with 2 µCi/ml L-¹⁴Cacetate for 1 h, washed once with PBS, and then incubated with 1.5  N NaOH for 10 min before transfer into chloroform tubes. The monolayers were washed with 10% SDS containing 70 °C 1.5 h. The unsaponified lipids were extracted with petroleum ether, and the upper phase was dried under a stream of N₂ at 45 °C. The lipids were resuspended with ethanol:acetone (1:1) and then the sterols precipitated by the addition of 1% digitonin. The precipitates were washed twice with acetone and counted in a scintillation counter.

**Phosphorysine Analysis**—HepG2 cells were plated into six-well plates at 4 × 10⁵ cells/well. After 48 h (about 70% confluence), the cells were incubated with various agents for the indicated time, rapidly washed once with ice-cold PBS, and lysed with 100 µl of SDS sample buffer containing 50 mM 2-mercaptoethanol and 500 µM sodium vanadate. The supernatants were subjected to electrophoresis on 7.5% gels. Gels were dried and autoradiographed toethanol, boiled for 5 min, and then separated by polyacrylamide gel electrophoresis on 7.5% gels. Gels were dried and autoradiographed on Kodak X-AR5 film.

**RESULTS**

The effects of oncostatin M on LDL uptake in HepG2 cells cultured in media that up-regulated (LPDS) or down-regulated (fetal bovine serum) LDL receptors were investigated. Oncostatin M, at 100 ng/ml (4 ng/ml) stimulated LDL uptake approximately 60% over the fetal bovine serum controls (Fig. 2A). The concentration of oncostatin M required to give a half-maximal increase was 3–5 ng/ml (0.1–0.2 nm); at concentrations of 50 ng/ml or higher a maximum stimulation of 70% was achieved (Fig. 2B).

To determine whether the LDL uptake stimulation was caused by increased receptor number, we employed an LDL receptor ELISA using the C-7 antibody (21). Fig. 2C reveals that oncostatin M increased LDL receptor immunoreactivity with a time dependence and magnitude that are similar to those of the LDL uptake response. Cycloheximide completely abolished the up-regulation induced by oncostatin M (Fig. 1).
Oncostatin M Up-regulates Liver LDL Receptors

**Fig. 2. Effects of oncostatin M LDL receptors.** The data in A and B represent means and S.E. from three or more experiments. A, LDL uptake time dependence. Oncostatin M (100 ng/ml) was incubated with HepG2 monolayers cultured in medium containing LPDS for the indicated times before assaying for LDL uptake. Oncostatin M was added at different times to assay LDL uptake simultaneously for the indicated times before assaying for LDL uptake. Oncostatin M was added at different times to assay LDL uptake simultaneously for the indicated times before assaying for LDL uptake. Oncostatin M (100 ng/ml) was incubated with HepG2 LDL receptors by stimulating cell proliferation (24). To determine whether the oncostatin M effects on LDL receptor were linked to cholesterol synthesis inhibition, we examined the effects of the oncostatin M receptor on second messenger pathways. Within 2 min after the addition of oncostatin M, a 20% increase in diacylglycerol was detected (Fig. 4A). The stimulation reached 50% by 30 min and remained elevated for at least 60 min. To investigate whether the elevated diacylglycerol arose from induced degradation of phospholipids, we studied the effects of oncostatin M on phospholipid metabolism. Within 15 min the polypeptide increased 32P incorporation into phosphatidylcholine by 50% and into the phosphoinositides by 25% (Fig. 4B).

**Table I**

*Effect of oncostatin M on HepG2 sterol and DNA synthesis*

For sterol synthesis studies, cells incubated in LPDS media were treated with 100 ng/ml oncostatin M for the indicated times. One h before the end of the experiments, [14C]acetate (2 μCi/ml) was added, and incorporation of radiolabel into nonapoformipinid lipids was determined as described under “Materials and Methods.” DNA synthesis was estimated by incorporation of radiolabeled thymidine in cells treated with the indicated concentrations of oncostatin M for 24 h as outlined under “Materials and Methods.” The cpm represent the means from several different experiments (indicated by the number in parentheses).

| Sterol synthesis | DNA synthesis |
|------------------|---------------|
| Incubation time  | cpm | % Change | Concentration | cpm | % Change |
| h                | ng/ml |          | h            | ng/ml |          |
| 0                | 18,600 (4) | 0        | 0            | 69,300 (2) | -9      |
| 2                | 18,300 (4) | -2       | 10           | 62,800 (2) | -15     |
| 4                | 18,503 (3) | -1       | 100          | 58,450 (2) | -15     |
| 8                | 18,697 (4) | +1       | 200          | 61,100 (2) | -12     |
| 20               | 8,439 (3)  | -55      |              |       |         |

**Fig. 3. Cross-linking of 125I-labeled oncostatin M (OM) to a HepG2 cell surface protein.** Confluent monolayers of HepG2 or H2981 cells were incubated with 4 nM 125I-oncostatin M alone (−) or radiolabeled oncostatin M plus 400 nM unlabeled oncostatin M (+) for 3 h. The cell proteins were then cross-linked and analyzed as described under “Materials and Methods.” Oncostatin M receptor-positive cells (H2981) were used as controls. cross-linked to the cell surface. 125I-oncostatin M was associated with a high molecular mass protein (approximately 150 kDa); this association was inhibited in the presence of unlabeled oncostatin M (Fig. 3). A cell line known to possess a high density of oncostatin M receptors (H2981; Ref. 15) was used as a positive control.

Since HepG2 cells appeared to possess a specific oncostatin M receptor, we examined the effects of the protein on second messenger pathways. Within 2 min after the addition of oncostatin M, a 20% increase in diacylglycerol was detected (Fig. 4A). The stimulation reached 50% by 30 min and remained elevated for at least 60 min. To investigate whether the elevated diacylglycerol arose from induced degradation of phospholipids, we studied the effects of oncostatin M on phospholipid metabolism. Within 15 min the polypeptide increased 32P incorporation into phosphatidylcholine by 50% and into the phosphoinositides by 25% (Fig. 4B).

The oncostatin M-induced increase in diacylglycerol suggested that the signal transduction pathway involved activation of protein kinase C. Treatment of HepG2 cells with phorbol myristate acetate (50 nM) for 4 h produced a 40% increase in LDL uptake, and this increase was inhibited by pretreatment of the cells with the protein kinase C inhibitor H-7. Furthermore, H-7 completely blocked the stimulation of HepG2 LDL uptake induced by 4 h of incubation with oncostatin M (see Table III).
Recently, it has been reported that diacylglycerol levels can be elevated by a mechanism that includes tyrosine kinase-mediated activation of phospholipase C-γ1 (26). The possibility that a tyrosine kinase could be activated by oncostatin M was investigated. Oncostatin M induced increases in tyrosine phosphorylation of several proteins, including a 165/180-kDa doublet and two other heavier bands at 110 and 125 kDa (Fig. 5C and Fig. 6). Phosphorylation could be detected at low concentrations of 1–5 ng/ml. At optimal concentrations (25–50 ng/ml), phosphorylation of the doublet at 165/180 kDa increased 5–10-fold over unstimulated cells whereas phosphotyrosine content in the bands at 110 and 125 kDa increased 2–3-fold (Fig. 5, A and C). Phosphorylation of the 165/180 kDa doublet occurred very rapidly. It was detectable after 1 min (the earliest time point taken), peaked between 3–5 min, and then decreased over the next 25–30 min (Fig. 5, B and C). Phosphorylation of other bands (80 and 95 kDa) remained elevated for at least 30 min (Fig. 5C). As indicated under "Materials and Methods," phosphorylation was measured by Western blotting techniques using anti-phosphotyrosine antibodies. Phenylphosphate or phosphotyrosine (10 mM) completely blocked binding of the antibody to nitrocellulose; phosphoserine had no effect (data not shown).

The effects of other growth factors known to stimulate tyrosine kinase activity on both tyrosine phosphorylation and LDL receptors in HepG2 cells were assessed (Fig. 6 and Table II). Although insulin (10 μg/ml) induced the phosphorylation of a new band at about 160 kDa and enhanced phosphorylation of the bands at 110 and 125 kDa, it had only minor effects on LDL uptake. EGF (100 ng/ml) stimulated the phosphorylation of a band at 175 kDa and enhanced phosphorylation of the 110- and 125-kDa bands. PDGF also stimulated LDL uptake by approximately 40%. Treatment with PDGF (10 ng/ml) gave no observable increase in tyrosine phosphorylation or LDL uptake. EGF or insulin, but not PDGF, gave rise to small increases in DNA synthesis (Table II).

Since the results suggested an early role for tyrosine kinase activity in oncostatin M-induced changes, the effects of the tyrosine kinase inhibitor genistein (27) were investigated. Pretreatment of HepG2 cells with genistein inhibited in-
TABLE II
Effects of various polypeptides on HepG2 cells

HepG2 cells were treated with the indicated factors and assayed as described under "Materials and Methods." Total incubation time was 6 h for LDL uptake and 24 h for DNA synthesis. LDL uptake values are shown as percent stimulation of control uptake (245 ng of LDL/mg of cell protein) in LPDS media. DNA synthesis values represent percent change compared with control data (control incorporation is shown in Table I). The number of experiments is shown under n.

| Polypeptide       | LDL uptake | DNA synthesis |
|-------------------|------------|---------------|
|                   | ng/mg      | % Stimulation | % Change |
| Oncostatin M      | 419        | +71           | 4         | -15 2 |
| Insulin           | 289        | +18           | 3         | +11 2 |
| EGF               | 348        | +42           | 4         | +6   2 |
| PDGF              | 246        | 0             | 2         | 0    2 |

TABLE III
Modulation of oncostatin M effects on HepG2 cells by selected agents

LDL uptake stimulation was based on control uptake values (ng of LDL/mg of cell protein). Genistein alone caused a significant decrease in LDL uptake. Values for tyrosine phosphorylation were from densitometry of the 165/180 doublet (Fig. 6) and represent fold increases compared with the control values, which were arbitrarily set at 1. Diacylglycerol data were calculated as percent stimulation compared with incorporation of radiolabel in control cells. Cells were pretreated with genistein (30 µg/ml), vanadate (50 µM) or H-7 (10 µg/ml) for 5 h (tyrosine phosphorylation) or for 30 min before the addition of oncostatin M (100 ng/ml) or PMA (50 nM). Oncostatin M incubation time was 6 h for LDL uptake, 5 min for tyrosine phosphorylation, and 30 min for diacylglycerol levels. ND indicates not determined.

| Polypeptide       | LDL uptake | Tyrosine phosphorylation | Diacylglycerol stimulation |
|-------------------|------------|--------------------------|---------------------------|
|                   | ng/mg      | %                        | %                         |
| Unstimulated      | 245        | 0                        | 0                         |
| Oncostatin M      | 429        | +75                      | 8                         | +53 |
| Genistein + M     | 270        | +10                      | 4                         | -1  |
| Vanadate + M      | 311        | +27                      | >20                       | +10 |
| Vanadate + M      | 541        | +121                     | >20                       | +65 |
| PMA               | 343        | +40                      | 1                         | ND  |
| Genistein + PMA   | 319        | +30                      | ND                        | ND  |
| H-7               | 221        | -10                      | ND                        | ND  |
| H-7 + M           | 265        | +8                       | ND                        | ND  |

Increases in tyrosine phosphorylation, in LDL uptake, and diacylglycerol stimulated by oncostatin M (Table III). The effects of genistein were not caused by general cytotoxicity since the inhibition was reversed by washing genistein from the cells prior to stimulation and also because the inhibitor had no effect on HepG2 DNA synthesis over 24 h. In addition, genistein only slightly inhibited LDL uptake stimulated by treatment of the cells with phorbol myristate acetate.

In contrast to these results, the phosphotyrosine phosphatase inhibitor sodium orthovanadate enhanced the effect of oncostatin M on LDL uptake (Table III) although this required 2-4 h of preincubation with vanadate. In control HepG2 cells treated with sodium vanadate, tyrosine phosphorylation was enhanced greatly. The vanadate effect was so marked, however, that it was difficult to identify oncostatin M-induced tyrosine phosphorylation in the presence of vanadate. Alone, the phosphatase inhibitor only partially increased LDL uptake (Table III).

DISCUSSION

Agents that increase LDL receptors by the sterol regulatory pathway (i.e., competitive inhibitors of cholesterol synthetic enzymes) first act to decrease the concentration of intracellular cholesterol. This reduction in sterols causes derepression of the LDL receptor gene, and transcription occurs (24). In the present study we show that the polypeptide oncostatin M is recognized by specific receptors on the surface of a liver cell line and rapidly up-regulates LDL receptors. Oncostatin M does not appear to act via the sterol-dependent pathway since up-regulations of similar magnitude were induced by the factor both in the presence and absence of exogenous cholesterol. In addition, both LDL uptake and the number of LDL receptors were maximally up-regulated (4-8 h) whereas effects on cholesterol synthesis were not yet detected. Thus oncostatin M apparently up-regulates the expression of the LDL receptors by a novel mechanism.

We have shown that within 1-2 min after treatment of HepG2 cells with oncostatin M there is a large increase in the levels of phosphotyrosine of several proteins. Pretreatment of the cells with the phosphotyrosine kinase inhibitor genistein partially blocked both tyrosine phosphorylation and LDL receptor up-regulation induced by the peptide. Additionally the phosphotyrosine phosphatase inhibitor vanadate increased both the levels of tyrosine phosphorylation and oncostatin M-induced LDL uptake. These results suggest a role for oncostatin M-induced tyrosine kinase activation in the LDL receptor up-regulation mechanism.

In addition to tyrosine kinase, protein kinase C may be involved in the pathway in which oncostatin M induces LDL receptor up-regulation. It has been reported that tyrosine kinase activation by other peptide factors leads to the activation of phospholipase C-γ1 by phosphorylation of a tyrosine residue (26). Our data support a similar mechanism for oncostatin M. The rapid rise in diacylglycerol levels and the phospholipid metabolism alterations are consistent with phospholipase C activation. Protein kinase C involvement is likely, based on the increase in diacylglycerol, the finding that the protein kinase C inhibitor (H-7) prevented the up-regulation, and the ability of the phorbol ester activator of protein kinase C to increase LDL uptake. This last result extends the observation by others that protein kinase C activators increase the message levels for the LDL receptor in HepG2 cells (29).

From these findings it is tempting to speculate that oncostatin M stimulates a tyrosine kinase which then phosphorylates and activates a phospholipase. The inhibition of production of diacylglycerol by pretreatment with genistein, but the lack of effect of genistein on phosphor myristate acetate induction of LDL receptors, indicates that tyrosine kinase activation precedes the generation of diacylglycerol. Diacylglycerol activates protein kinase C, which in turn induces LDL receptor expression, presumably by stimulation of transcription. Protein kinase C activation may also explain the delayed onset of cholesterol synthesis inhibition which occurs by 20 h of incubation. It has been reported that phosphorylation of the rate-limiting enzyme for cholesterol synthesis (hydroxymethylglutaryl-coenzyme A reductase; EC 1.1.1.88) by protein kinase C inhibits its activity (28).

The effects of several factors that are known to stimulate tyrosine kinase activation were compared in HepG2 cells. Epidermal growth factor (EGF) stimulated the phosphorylation of peptides of 110, 125, and 175 kDa and caused increases in LDL uptake. Insulin increased tyrosine phosphorylation but had no effect on LDL uptake. Since not all peptides that stimulate tyrosine kinase activity up-regulated the LDL receptor, oncostatin M must stimulate the activity of a specific kinase or the phosphorylation of a specific substrate that is involved in regulating the levels of the LDL receptor. The identities of the oncostatin M-stimulated tyrosine kinase and

18198 Oncostatin M Up-regulates Liver LDL Receptors
Oncostatin M up-regulates liver LDL receptors.

It is intriguing to note that the liver has a large number of macrophage-derived Kupffer cells residing in close proximity to the parenchymal cells where secretory factors would be especially accessible to the hepatocyte.

Although regulation of oncostatin M secretion is not understood, the presence of oncostatin M in media from macrophages stimulated with endotoxin (13) indicates that endotoxin can increase oncostatin M production. Identification of other stimulators of oncostatin M secretion by macrophages is currently in progress.

REFERENCES
1. Goldstein, J. L., and Brown, M. S. (1977) Annu. Rev. Biochem. 46, 897-930
2. Brown, M. S., and Goldstein, J. L. (1986) Science 232, 34-47
3. Kovanen, P. T. (1987) Am. Heart J. 113, 644-649
4. Yokode, M., Hammer, R. E., Ishibashi, S., Brown, M. S., and Goldstein, J. L. (1990) Science 250, 1273-1275
5. Molowa, D. T., and Cimis, G. M. (1989) Biochem. J. 260, 731-736
6. Russel, D. W., Yamamoto, T. L., Schneider, W. J., Slaughter, C. J., Brown, M. S., and Goldstein, J. L. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 7501-7505
7. Hui, D. Y., Innerarity, T. L., and Mahley, R. W. (1981) J. Biol. Chem. 256, 5646-5655
8. Spady, D. K., and Dietschy, J. M. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4526-4530
9. Nathan, C. F. (1987) J. Clin. Invest. 79, 319-326
10. Nemer, S. D., Champlin, R. E., and Golde, D. W. (1988) J. A. M. A. 260, 3297-3300
11. Garraik, M. B., and Stoudemire, J. (1989) Clin. Res. 37, 260 (abstr.)
12. Cai, H-J., He, Z-G., and Ding, Y-N. (1988) Biochim. Biophys. Acta 959, 334-342
13. Grove, R. I., Mazzucco, C. E., Radka, S. F., and Shoyab, M. (1990) Circ. Res. 82 (Suppl. III), 326 (abstr.)
14. Zarling, J. M., Shoyab, M., Marquardt, H., Hanson, M. B., Lioubin, M. N., and Todaro, G. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 9759-9763
15. Linsley, P., Bolton-Hanson, M., Horn, D., Malik, N., Kallestad, J., Ochs, V., Zarling, J. M., and Shoyab, M. (1989) J. Biol. Chem. 264, 4282-4289
16. Kamps, M. P., and Selton, B. M. (1968) Oncogene 2, 305-315
17. Pitas, R. E., Innerarity, T. L., Weinstein, J. N., and Mahley, R. W. (1981) Arterioscler. 1, 177-185
18. Reynolds, G. D. (1985) Surg. Pathol. Res. 4, 389-400
19. Kingsley, D. M., and Krieger, M. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 5454-5458
20. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911-917
21. Beisiegel, U., Schneider, W. J., Goldstein, J. L., Anderson, R. G. W., and Brown, M. S. (1981) J. Biol. Chem. 256, 11923-11931
22. Mosley, S. T., Kalinowski, S. S., Schafer, B. L., and Tanaka, R. D. (1989) J. Lipid Res. 30, 1411-1417
23. Grove, R. I., Allegretto, N. J., Kiener, P. A., and Warr, G. A. (1990) J. Leukocyte Biol. 48, 38-42
24. Bilheimer, D. W., Grundy, S. M., Brown, M. S., and Goldstein, J. L. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 4124-4128
25. Chait, A., Ross, R., Albers, J. L., and Bieman, E. L. (1986) Proc. Natl. Acad. Sci. U. S. A. 77, 4084-4088
26. Todderud, G., Wahl, M. I., Rhee, S. G., and Carpenter, G. (1990) Science 249, 296-298
27. Miettinen, T., Cogheshall, K. M., Isakov, N., and Altman, A. (1990) Science 247, 1584-1587
28. Beg, Z. H., Reznikov, D. C., and Avigan, J. A. (1986) Arch. Biochem. Biophys. 244, 310-322
29. Auwerx, J. H., Chait, A., Wolfbauer, G., and Deeb, S. S. (1989) Mol. Cell. Biol. 9, 2298-2302