Multilevel Regulation of Low-Density Lipoprotein Receptor and 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Gene Expression in Normal and Leukemic Cells

By Sigurd Vitols, Svante Norgren, Gunnar Julusson, Loukas Tatidis, and Holger Luthman

Altered cholesterol homeostasis has been noted in malignant cells, which led us to explore the regulation of cholesterol metabolism in normal and leukemic cells. The mean low-density lipoprotein (LDL) receptor and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activities were fivefold and threefold higher in mononuclear blood cells from 33 patients with leukemia, compared with cells from 23 healthy subjects, whereas elevations in RNA levels were twofold and 40% only. The activities of the two proteins correlated in normal cells (r = .46), whereas an inverse correlation was found in leukemic cells (r = -.40). Relatively weak correlations were found between LDL receptor RNA levels and receptor activity in normal (r = .48) and leukemic cells (r = .49), and HMG-CoA reductase RNA levels correlated (r = .53) with reductase activity in leukemic cells only. The ratios of protein activities to RNA levels in cells were constant during consecutive blood samplings and similar in leukemic blood and bone marrow cells from the same individual. During cholesterol deprivation, protein activities increased more than RNA levels, and leukemic cells with high LDL receptor activity showed a partial resistance to the suppressing effect of sterols on LDL receptor gene expression. The results demonstrate that LDL receptor RNA levels alone cannot explain variation in receptor activity, suggesting post-RNA regulation of LDL receptor expression, similar to what has been described for HMG-CoA reductase. Taken together, the present results suggest multilevel regulation of both proteins and demonstrate that each cell clone, normal or malignant, has a unique ratio of protein activity to RNA level. Leukemic cells, in contrast to normal cells, can meet increased cholesterol requirements by either elevated LDL receptor activity or increased cholesterol synthesis, which is of potential interest for diagnosis and specific treatment of leukemia.

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Mammalian cells need cholesterol for synthesis of cell membranes, steroid hormones, and bile acids. The requirements for cellular cholesterol are met by receptor-mediated uptake of preformed cholesterol in low-density lipoprotein (LDL) and/or by endogenous synthesis, the rate limiting enzyme in the synthesis pathway being 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. LDL is the major cholesterol-carrying lipoprotein in human plasma, containing approximately 70% of total plasma cholesterol. Each LDL particle is composed of a lipid core of about 1,500 molecules of cholesteryl ester surrounded by a polar shell of phospholipids, free cholesterol, and apolipoprotein (apo) B. The latter constitutes the receptor recognizing function of the particle. Once bound to the LDL receptor, LDL is internalized and degraded in lysosomes. The released cholesterol is available for the cells use or stored in reesterified form.1

When deprived of cholesterol, cultured cells respond by increasing endogenous sterol synthesis and LDL receptor activity as well as by expressing elevated levels of mRNAs encoding the HMG-CoA reductase and the LDL receptor respectively.2,4 Conversely, if LDL or sterols are added to cholesterol-deprived cells, sterol synthesis and LDL receptor activity decrease and the two mRNAs are suppressed. Transcriptional regulation of the LDL receptor gene has been shown to depend on the presence of three imperfect direct repeats of 16 bp and a TATA-like sequence in the 5’ flanking region of the gene.3 Repeats 1 and 3 have been shown to interact with the general positive transcription factor Sp1, whereas repeat 2 containing an 8-bp segment, designated sterol regulatory element-1 (SRE-1), is believed to interact with another enhancing protein that influences the action of Sp1. Inactivation of this enhancing protein in the presence of sterols would account for the sterol-mediated decline in transcription.3 For the HMG-CoA reductase gene, also post-transcriptional control mechanisms such as changes in translational efficiency, degradation of protein, and inactivation of enzyme have been established in addition to sterol-mediated regulation of transcription.3 However, little is known about the regulation of these two proteins in different cells in vivo. There are indications of differences in the regulation of cholesterol metabolism in normal and malignant cells because previous studies have shown that freshly isolated leukemic cells from patients with acute myelogenous leukemia (AML) have elevated receptor-mediated uptake and degradation rates of LDL, as compared with white blood and bone marrow cells from healthy individuals.5,6 Recently 14C-sucrose-labeled LDL administered intravenously (IV) to patients with acute leukemia confirmed that the LDL receptor activity of AML cells is elevated also in vivo.7 To further investigate the regulation of cholesterol metabolism in normal and malignant cells, we compared the regulation of LDL receptor and HMG-CoA reductase gene expression in mononuclear blood cells from healthy individuals with leukemic cells from patients with different types of leukemia.

MATERIALS AND METHODS

Patients with leukemia and normal subjects. Blood and/or bone marrow samples were analyzed from 34 patients (19 men and 15 women) and 34 healthy donors.

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women) with different types of leukemia. Bone marrow only was obtained from 1 patient with AML. The patients represent consecutive cases studied in the laboratory but not necessarily consecutive admissions. The study included 1 patient with acute lymphoblastic leukemia (ALL), 27 with AML (21 de novo, 4 secondary, 2 relapsed), 1 with chronic myelogenous leukemia (CML), 2 with CML in blast crisis, 2 with chronic lymphocytic leukemia (CLI), and 1 patient with a myelodysplastic syndrome (MDS). The ages of the patients with different leukemia subgroups were ALL, 45 years; AML, mean 66 years (range, 41 to 85 years); CML, 33 years; CML in blast crisis, 65 and 72 years; CLI, 67 and 76 years; MDS, 21 years. Leukemias were classified according to the French-American-British (FAB) subclassification system. The mean white blood cell count was 106 × 10⁹/L (range, 4 to 800 × 10⁹/L) and the mean fraction of leukemic cells in peripheral blood was 74% (range, 29% to 100%). The mean total and LDL cholesterol values were 3.5 (range, 1.0 to 6.4) and 2.6 (range, 0.5 to 5.4) mmol/L, respectively (n = 29). The healthy subjects, who were blood donors or laboratory staff members at the Karolinska hospital, consisted of 23 individuals (13 males, 10 females); their mean age was 39 years (range, 21 to 64). A 42-year-old man had a total plasma cholesterol and LDL cholesterol value of 9.2 and 7.3 mmol/L, respectively. Approval for the study was obtained from the local ethics committee.

**Materials.** Sodium 125I (carrier free, pH 7 to 11), cytidine 5'-[α-125I]thiotriphosphate (tritylaminonium salt, > 1,000 Ci/mmol), uridine 5'-[α-125I]thiotriphosphate (tritylaminonium salt, > 1,000 Ci/mmol), 3-hydroxy-3-methyl-[125I]coenzyme A (52 mCi/mmol), and DL-(2-H) mevalonic acid lactone (1.26 Ci/mmol) were from Amersham (U.K.). RNase A, RNase T1, proteinase K, transfer-RNA, and salmon sperm DNA were obtained from Sigma Chemical Co (St. Louis, MO). Formamid (Pro Analysis [pa]) was from Merck (Darmstadt, Germany) and was used after recrystallization. The plasmids, pGEM-4Z, pGEM-SF1 (+), restriction endonucleases, and the in vitro transcription kit (Riboprobe Gemini II Core System) were from Promega Biotec (Madison, WI). Sephadex G-50 columns (Nick columns) were from Pharmacia (Uppsala, Sweden). Mevinolin was provided by Dr A.W. Alberts (Merck Sharp & Dome, Rahway, NJ) and 25-hydroxycholesterol was from Steraloids, Inc (Wilton, NH).

**Solutions.** Mevinolin in the lactone form was converted to the sodium salt by heating at 50°C for 1 hour in 0.1 mol/L NaOH, after which the solution was neutralized with 1 mol/L HCl and stored in multiple aliquots at −20°C.

**Lipoproteins.** LDL (density, 1.020 to 1.063 g/mL) and human lipoprotein-deficient serum (LPDS; density, > 1.215 g/mL) were isolated from serum of healthy blood donors by sequential ultracentrifugation. The purity of LDL and LPDS preparations were examined by agarose gel electrophoresis, and the absence of cholesterol in LPDS was confirmed by enzymatic cholesterol analysis (Merck). 125I-Labeled LDL (specific activity, 140 to 260 cpm/ng protein) was prepared as described by Langer et al. Less than 1% of the radioactivity in the 125I-LDL preparations was present as free iodide. The concentrations of LDL refer to protein.

**Cell isolation and incubations.** Mononuclear cells were isolated from peripheral blood or bone marrow by centrifugation on Lymphoprep (density, 1.077 g/mL) and washed with ice-cold phosphate-buffered saline (PBS) 140 mmol/L NaCl, 2.7 mmol/L KCl, 9.5 mmol/L Na₂HPO₄, and 9.5 mmol/L KH₂PO₄, pH 7.4, as described earlier. An aliquot of the washed cells was used for immediate determination of LDL receptor activity, as described below. The remainder of the cells were centrifuged and pellets were frozen in liquid nitrogen for later determinations of HMG-CoA reductase activity (2 to 3 × 10⁹ cells) and RNA levels (20 to 40 × 10⁹ cells).

In some experiments, cells were subjected to incubation in RPMI 1640 medium containing 10% LPDS with and without different additions, as indicated. The cell concentration was adjusted to 2 × 10⁶ cells/mL, and 35-mL portions were incubated in 162-cm² cell culture flasks (Costar Co, Cambridge, MA) at 37°C. After different time periods, flasks were removed, the cells were recounted, and LDL receptor activity was determined in aliquots of the cell suspension. The remainder of the cells were washed twice with ice-cold PBS, and pellets were frozen for later HMG-CoA determination and RNA analysis. If LDL or sterols were present during the preincubations, the cells (including controls) were washed (as above) before determination of LDL receptor activity.

**LDL receptor and HMG-CoA reductase activity determinations.** The high-affinity (receptor-mediated) degradation rate of 125I-LDL was used as a measure of LDL receptor activity. In brief, 3 × 10⁶ cells (2 × 10⁶ if cells were subjected to preincubation) were incubated at 37°C in 1 mL of RPMI 1640 supplemented with 10% LPDS with 25 μg of 125I-LDL in the absence or presence of a 20-fold excess of unlabelled LDL. Cellular degradation of 125I-LDL was determined from the formation of acid-soluble radioactivity in the incubation medium. The high-affinity degradation rate was calculated by subtracting the degradation of 125I-LDL in the presence of excess unlabelled LDL (unspecific degradation) from the degradation in the absence of unlabelled LDL (total degradation). HMG-CoA reductase activity in cell-free extracts was determined from the rate of conversion of 3-hydroxy-3-methyl-[14C]glutaryl coenzyme A (25,000 dpm/mmol) to 14C-mevalonate in detergent-solubilized extracts. HMG-CoA reductase activity is expressed as picomoles of 14C-mevalonate formed per minute per milligram of detergent-solubilized protein. Protein concentration was determined with bovine serum albumin as a standard.

**RNA analysis.** Total nucleic acid (TNA) extracts were prepared essentially as described. Between 5 and 10 × 10⁹ mononuclear cells were lysed per milliliter of 1× SET (1% sodium dodecyl sulfate [SDS], 20 mmol/L Tris-HCl, pH 7.5, and 10 mmol/L EDTA) and homogenized with a Polytron (Kinematica Typ PT 16/35; Kriens, Luzern, Switzerland) for 10 to 15 seconds at setting 5 to 6, whereafter proteinase K was added to a final concentration of 0.2 mg/mL and the homogenate was incubated for 45 minutes at 45°C. After phenol/ chloroform extraction, ethanol precipitation, and centrifugation, the pellets were dissolved in 0.2× SET. Aliquots were taken for determination of DNA concentration by Hoechst fluorometry.

A 265-bp Pst I fragment, (nucleotides 1,066 to 1,330) of the cDNA clone pLDLR-2 from the American Type Culture Collection, Rockville, MD) encoding the human LDL receptor, was subcloned into the Pst I site of PGEM-4Z. The resulting vector, pLDR-265, was sequenced and used for in vitro transcription as described by Melton et al¹³ and the supplier. For synthesis of the labeled antisense probe, pLDR-265 was cleaved with BamHI and transcribed with T7 RNA polymerase in the presence of [α-35S]UTP and [α-35S]CTP. The 309-bp antisense RNA was separated by unincorporated nucleotides by filtration on a Sephadex G-50 column. Polyacrylamide gel electrophoresis of the probe and subsequent autoradiography of dried gels showed that approximately 90% of the probe was of the expected size. A 324-bp unlabeled sense RNA was transcribed with SP6 RNA polymerase from HindIII-cleaved pLDR-265. After Sephadex G-50 chromatography, the RNA-containing fractions were pooled and ethanol precipitated. Finally, the pellet was dissolved in 0.2× SET and the concentration of the unlabeled RNA was determined spectrophotometrically, assuming that a 40 µg/mL RNA solution has an absorption of 1 at 260 nm. To obtain a probe for human HMG-CoA reductase RNA, a 50-bp oligonucleotide corresponding to nucleotides 1254 to 1303 was synthesized with a BamHI-linker on an Applied Biosystem (Foster City, CA) DNA synthesizer. The oligonucleotide was subcloned into the BamHI site of pGEM-4Z, and the

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resulting vector was sequenced. After cleavage with HindIII, in vitro transcription was performed with SP6 RNA polymerase in the presence of [35S]-UTP and [35S]-CTP. More than 90% of the probe was of the expected size (113 bp). In vitro transcription of EcoRI-cleaved vector with T7 RNA polymerase generated the corresponding sense (120,000 bp). In vitro transcription of EcoRI-cleaved LDL receptor RNA. The 119-bp transcript was purified, and the concentration was determined spectrophotometrically.

Hybridizations were performed as described.17-19 Aliquots of unlabeled sense RNA or TNA extracts containing 5 to 40 μg of DNA in 20 μL 0.2× SET were mixed with approximately 30,000 cpm of the probe in 20 μL of hybridization solution (0.6 mol/L NaCl, 4 mmol/L EDTA, 7.5 mmol/L dithiotreitol, 50% recrystallized formamide, 20 mmol/L Tris-HCl, pH 7.5) and incubated at 68°C. After 18 hours, 1 mL of RNase solution (40 μg/mL RNase A, 2 μg/mL RNase T1, 100 μg/mL salmon sperm DNA, 0.3 mol/L NaCl, 2 mmol/L EDTA, 10 mmol/L Tris-HCl, pH 7.5) and incubated at 37°C for 45 minutes. The RNase treatment was terminated by the addition of 100 μL ice-cold TCA (100%). After 30 minutes on ice, the precipitates were collected on GF/C filters (Whatman International Ltd, Maidstone, UK) and washed extensively with 4% TCA. Finally, 8 mL of Instagel (Packard Instrument Co, Downers Grove, IL) was added, and the radioactivity on the filters was determined in a liquid scintillation counter (Packard). Blank values were below 0.4% of the input radioactivity as determined from hybridizations without sense RNA or TNA but otherwise identically treated. RNA levels in the TNA extracts were quantified by comparing the hybridization signal with the linear part of the standard curve, which was generated by hybridizations with different concentrations of sense RNA. All RNA values are based on hybridizations of three serial dilutions of each extract and presented as RNA copies per diploid genome (6 pg DNA). The limit of detection, set as twice the background radioactivity, was equivalent to 0.10 and 0.20 copies/cell for the LDL receptor and HMG-

Table 1. LDL Receptor and HMG-CoA Reductase Gene Expression in Freshly Isolated Normal and Leukemic Cells

<table>
<thead>
<tr>
<th></th>
<th>Healthy Subjects (n = 23)</th>
<th>Leukemias (n = 23)</th>
<th>AML Only (n = 23)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL receptor</td>
<td></td>
<td></td>
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<tr>
<td>Degradation of [3H]-LDL (ng/10⁶ cells)</td>
<td>0.50 ± 0.18</td>
<td>2.46 ± 2.92†</td>
<td>2.80 ± 3.03†</td>
</tr>
<tr>
<td></td>
<td>(0.25-0.83)</td>
<td>(0.01-11.7)</td>
<td>(0.01-11.7)</td>
</tr>
<tr>
<td></td>
<td>RNA (copies/cell)</td>
<td>0.78 ± 0.34</td>
<td>1.52 ± 1.01†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.12-1.65)</td>
<td>(0.13-4.52)</td>
</tr>
<tr>
<td></td>
<td>HMG-CoA reductase</td>
<td>6.81 ± 2.89</td>
<td>20.04 ± 13.31†</td>
</tr>
<tr>
<td></td>
<td>Activity (pmol/min/mg)</td>
<td>(2.60-14.25)</td>
<td>(7.00-65.2)</td>
</tr>
<tr>
<td></td>
<td>RNA (copies/cell)</td>
<td>0.62 ± 0.30</td>
<td>0.92 ± 0.74†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.22-1.29)</td>
<td>(0.2-3.07)</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD (range).
* Including 2 patients with CML in blast crisis.
† P < .005 versus controls (Student’s unpaired t-test).
‡ P < .05 versus controls (Student’s unpaired t-test).

Fig 1. RNase protection experiments. (A) Lane 1, 6,000 cpm of the LDL receptor probe; lane 2, RNase resistant hybrid obtained by hybridization of 34,000 cpm of the LDL receptor with 14 pg of the corresponding in vitro synthesized sense RNA; lane 3, RNase resistant hybrid obtained from 8 pooled hybridizations of 34,000 cpm of the LDL receptor probe with TNA extracts containing 25 μg of DNA each obtained from leukemic cells with 1.4 LDL receptor RNA copies/cell. (B) Lane 1, 5,000 cpm of the HMG-CoA reductase probe; lane 2, RNase resistant hybrid obtained by hybridization of 120,000 cpm of the HMG-CoA reductase probe with 5 pg of the corresponding in vitro synthesized sense RNA; lane 3, RNase resistant hybrid obtained by hybridization of 120,000 cpm of the HMG-CoA reductase probe with total cellular RNA (37 μg) extracted from 20 x 10⁶ leukemic cells with 1.9 HMG-CoA reductase RNA copies/cell.
CoA reductase RNA assay, respectively, in samples containing 40 
µg of DNA. The coefficient of variation between identical extracts 
was 2.2% (n = 3) at a mean of 1.13 copies/cell of LDL receptor 
RNA and 12% (n = 3) at a mean of 0.78 copies/cell of HMG-CoA 
reductase RNA.

Validation of RNA analyses. The recovery of RNA in the final 
TNA extract was 70% to 80% (n = 4) as estimated from the recovery 
of in vitro labeled RNA [plasmid pGEM-5Zf(+) transcribed with 
SP6 RNA polymerase in the presence of 35S-UTP] added to cell 
samples during homogenization. For DNA, the recovery was 50% 
to 90% (n = 4) based on determinations of DNA concentrations by 
fluorometry immediately after homogenization and in the final TNA 
extract. The specificity of both probes was confirmed by an RNase 
protection assay using extracts from leukemic cells. The LDL receptor 
probe was hybridized with a TNA extract as described. After 
RNase treatment and extraction with phenol/chloroform, the samples 
were precipitated with ethanol and centrifuged. Finally, the samples 
were dissolved and analyzed by electrophoresis on a denaturing 5% 
polyacrylamide gel. Autoradiography of the dried gel demonstrated a 
major RNase resistant band of the expected size (Fig IA). For the 
HMG-CoA reductase probe, all steps were identical except that total 
RNA was extracted as described30 from leukemic cells instead of 
TNA. This minimized the problem of loading the gel with viscous 
samples. Also in this case, a major protected band of the expected 
size was observed (Fig IB). To rule out hybridization with DNA, 
both probes were hybridized under identical conditions with purified 
human genomic DNA. No RNase protected signal was observed 
when up to 100 µg of human genomic DNA was added.

Statistical methods. Regression lines were calculated according 
to the method of least squares. Student's t-test was used for test of 
significance and P values <.05 were considered significant.

RESULTS

Gene expression in freshly isolated cells. The mean val-
ues for high affinity degradation rate of 125I-LDL and HMG-
CoA reductase activity were approximately fivefold and 
threefold higher, respectively, in mononuclear blood cells 
from 33 patients with different types of leukemia compared 
with mononuclear blood cells from 23 healthy subjects (Ta-
ble 1). The elevations in corresponding RNA levels were 
less pronounced; they were twofold and 40% for the LDL 
receptor and HMG-CoA reductase, respectively. Table 1 also 
demonstrates the large interindividual variation in the ex-
pression of both genes among patients with leukemia.

LDL receptor RNA levels correlated with the degradation 
rates in normal cells (r = .48, P = .0202, Fig 2A) and in 
leukemic blood (r = .49, P = .0034, Fig 2B) and bone 
marrow cells (r = .87, P = .0001, n = 16, Fig 2B). Despite 
the overall correlations, the degradation rate showed a large 
variation between several individuals with similar LDL re-
ceptor RNA copy numbers. For HMG-CoA reductase, a sig-
nificant correlation was found between RNA levels and re-
ductase activity in leukemic cells from blood (r = .53, P = 
.0014, Fig 3) and bone marrow (r = .50, P = .0496, Fig 3), 
whereas no correlation was obtained in normal mononuclear 
cells (r = .05, P = .8285). Analysis of the relationships 
between the activities of the two proteins showed a differ-
ce between normal and leukemic cells; a positive correla-
tion was found between the degradation rate and HMG-CoA 
reductase activity in normal mononuclear cells (r = .46, P 
= .026, Fig 4A), whereas an inverse correlation was found 
in leukemic blood cells (r = -.40, P = .0198, Fig 4B). The 
levels of the two RNAs correlated in normal cells (r = .57, 
P = .0046), whereas no correlation was found in leukemic 
blood cells (r = .07, P = .71).

Paired blood and bone marrow leukemic cells were studied 
in 15 patients. In many patients, the absolute protein 
activities and RNA levels differed markedly in cells from 
the two body compartments. When the ratio of the degrada-
tion rate to the LDL receptor RNA level was plotted against 
the corresponding ratio in bone marrow cells (Fig 5), a sig-
significant correlation was obtained ($r = .73, P = .0021$). Also for HMG-CoA reductase, the correlation between the ratio of protein activity to RNA level in blood and bone marrow cells was significant ($r = .76, P = .0028$). Protein activities and RNA levels were also determined in mononuclear blood cells during repeated samplings. The ratio of protein activity to RNA level was constant at repeated determinations for both proteins in both normal and leukemic cells (Table 2). This was the case even if the period of time between samplings was extended to several months.

Effects of cholesterol deprivation on gene expression. To investigate the regulation of LDL receptor and HMG-CoA reductase gene expression during cholesterol deprivation, cells were subjected to prolonged incubations in lipoprotein-deficient medium. Figure 6A and B demonstrate typical results obtained from incubation of mononuclear cells from a healthy individual. During incubation in medium with 10% LPDS, there were rapid and simultaneous increases in both RNAs with peak levels reached after about 20 hours. The elevations in protein activities were delayed and more pronounced. As compared with zero time, the LDL receptor RNA level increased approximately fourfold and HMG-CoA reductase RNA 50%, whereas the degradation rate increased more than 20-fold and reductase activity more than fivefold. Figure 7 demonstrates the effects of cholesterol deprivation in leukemic cells from 3 patients with AML. As in normal cells, there were rapid and simultaneous increases in both RNAs during incubation in lipoprotein-deficient medium. In the leukemic cells with high LDL receptor activity (Fig 7A and B), the relative increases in LDL receptor RNA levels were smaller than in normal cells or leukemic cells with low LDL receptor activity (Fig 7C). Furthermore, the increases in degradation rates during cholesterol deprivation were more rapid in leukemic cells with high degradation rates compared with normal cells. Disproportionately large increases of protein activities relative to RNA were observed for both proteins also in leukemic cells. LDL receptor RNA levels increased 60%, twofold, and sixfold, whereas degradation rates increased threefold, 12-fold, and 17-fold, respectively, in the 3 patients. For HMG-CoA reductase, the largest discrepancy between elevations in RNA levels and protein activity was observed in cells from the second patient (Fig 7E). Here, RNA levels increased fourfold and reductase activity 16-fold. The same maximum LDL receptor RNA copy number (approximately, 8 copies/cell) was reached in cells
Table 2. LDL Receptor and HMG-CoA Reductase Gene Expression in Freshly Isolated Normal and Leukemic Cells During Consecutive Samplings

<table>
<thead>
<tr>
<th>Sampling (d)</th>
<th>WBC (×10⁶/L)</th>
<th>LDL Receptor</th>
<th>HMG-CoA Reductase</th>
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<tr>
<td></td>
<td></td>
<td>Degradation of [125I]LDL (ng/h/10⁶ cells)</td>
<td>Degradation/ RNA ratio</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RNA (copies/cell)</td>
<td></td>
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<tr>
<td>AML</td>
<td>0</td>
<td>11.7</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.74</td>
<td>1.13</td>
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<tr>
<td></td>
<td>5</td>
<td>13.2</td>
<td>2.35</td>
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<td>CML blast crisis</td>
<td>0</td>
<td>1.03</td>
<td>0.71</td>
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<tr>
<td></td>
<td>2</td>
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<td>AML resistant disease</td>
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<td>63</td>
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<td>Healthy subject</td>
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<td>1.65</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>10</td>
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<td>0.58</td>
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<tr>
<td>Healthy subject</td>
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<td>1.47</td>
<td>0.64</td>
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<tr>
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<td>10</td>
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<td>0.46</td>
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<tr>
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<td></td>
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<td>0.49</td>
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<tr>
<td>Healthy subject</td>
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<td>0.81</td>
<td>0.57</td>
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<tr>
<td></td>
<td>482</td>
<td>0.52</td>
<td>0.73</td>
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Abbreviation: —, not determined.

from all three patients, whereas the maximum degradation rates were different. Also for HMG-CoA reductase RNA, the same maximum copy number (3 copies/cell) was reached in cells from the 3 patients. No cell growth occurred under these conditions and nonspecific increase in RNA did not take place because the ratio between total RNA and DNA remained constant.

**Regulation of gene expression by LDL or sterols.** The effect of LDL on gene expression was studied by incubating normal and leukemic cells in lipoprotein-deficient medium in the absence or presence of LDL. Protein activities and RNA levels were then determined and compared with zero time (directly after isolation from blood). These experiments were performed with cells from 2 healthy subjects, 2 patients with AML whose cells showed moderate increases in "11-LDL degradation rates at zero time, and 1 patient with ALL whose cells exhibited a very low degradation rate (Table 3). Cells from the ALL patient and 1 of the patients with AML had also very high HMG-CoA reductase activities. Inclusion of LDL in the medium suppressed the increases in LDL receptor and HMG-CoA reductase RNA levels and protein activities in normal and leukemic cells. Cells from the patient with ALL responded differently to cholesterol deprivation, with a predominant induction of HMG-CoA reductase activity and almost no induction of LDL receptor activity. Inclusion of mevinolin, a potent competitive inhibitor of HMG-CoA reductase, in the medium resulted in much higher protein activities. In cells from the AML patients, mevinolin caused approximately 100-fold higher HMG-CoA reductase activities as compared with zero time. Elevations of RNA levels were far less pronounced.

Table 4 demonstrates the ability of different concentrations of LDL or sterols to suppress, during incubation in lipoprotein-deficient medium, the increases in LDL receptor activity and RNA levels in normal mononuclear cells and in...
In this study, we found a relatively weak correlation between LDL receptor RNA levels and LDL receptor activity in freshly isolated normal and leukemic cells, which demonstrates that RNA levels alone cannot explain the variation in LDL receptor activity between different individuals. This finding suggests additional regulatory mechanisms at the "post-RNA" level, such as changes in translational efficiency or posttranslational regulation. "Post-RNA" regulatory mechanisms are further supported by the discordant increases in LDL receptor activity relative to the RNA level in both normal and leukemic cells during cholesterol deprivation. Additional support for "post-RNA" regulatory mechanisms for the LDL receptor have emerged from the studies of Sharkey et al. They demonstrated that the LDL receptor activity of receptor-deficient cells transfected with viral vectors containing cDNA for the LDL receptor but lacking the 5' sterol regulatory element still was sterol-responsive. Transgene LDL receptor mRNA levels in the cells were not influenced by sterols, suggesting that other mechanisms mediate sterol regulation of the transduced LDL receptor activity. We found an overall correlation, which was of similar strength to that of the LDL receptor, between the RNA level and the reductase activity in freshly isolated leukemic cells. In combination with the results of prolonged incubations in lipoprotein-deficient medium, these findings demonstrate the presence of "post-RNA" regulatory mechanisms also for HMG-CoA reductase. Indeed, detailed cell culture studies have demonstrated multivalent and multilevel regulatory mechanisms for HMG-CoA reductase, but this has previously not been established for the LDL receptor.

Of particular interest is, for both proteins, the similar intra-individual ratio of protein activity to RNA level in leukemic blood and bone marrow cells, and the constant ratios in normal and leukemic cells during consecutive blood samplings. These two observations, together with the weak correlations between RNA levels and protein activities in freshly isolated blood cells from different individuals, are consistent with a model in which each cell clone has a unique ratio between protein activity and RNA level, and the RNA level is of importance for the protein activity.

The correlation between LDL receptor and HMG-CoA reductase RNA levels and the correlation between the corresponding protein activities in freshly isolated mononuclear cells from healthy individuals suggest coordinate regulation of gene expression in these cells in vivo. This is further supported by previous in vitro studies of cultured cells and the current study demonstrating coordinate regulation, both at the RNA level and protein level, during cholesterol deprivation. Coordinate transient induction of the transcription of these two genes has been demonstrated in phorbol ester-treated cultured cells, suggesting involvement of protein kinase C in transcriptional regulation. The lack of correlation between the two RNA levels and the inverse correlation between the protein activities in freshly isolated leukemic blood cells suggest different regulation of cholesterol homeostasis in these cells. Cuthbert et al. have demon-
Fig 7. Effects of prolonged incubations in medium containing 10% LPDS on LDL receptor and HMG-CoA reductase gene expression in leukemic cells. Mononuclear blood cells were isolated from three patients with AML: a 78-year-old man (A and D), a 75-year-old woman (B and E), and a 73-year-old woman (C and F). High-affinity degradation rates of 125I-LDL and LDL receptor RNA levels (A, B, and C), HMG-CoA reductase activities and HMG-CoA reductase RNA levels (D, E, and F). Each protein activity value represents the mean of duplicate determinations and each RNA value represents the mean of triplicate hybridizations.

It was demonstrated, using actinomycin D, that the increase in LDL receptor mRNA in normal mononuclear cells incubated in lipoprotein-deficient medium is due to ongoing gene transcription and not due to RNA stabilization. As in the current study, they observed a rapid increase in LDL receptor mRNA after 2 hours of incubation. However, they found, in contradiction to our study, that neither LDL nor oxygenated sterols suppressed the increase in LDL receptor mRNA after 2 hours of incubation. We could demonstrate, probably due to the more sensitive and quantitative RNA method used, that this initial upregulation indeed is suppressed by both LDL or sterols in both normal and leukemic cells and that AML cells with high LDL receptor activity have a decreased sensitivity to regulation by exogenous sterols. The similar suppressing effect of LDL on LDL receptor gene expression in normal and leukemic cells and the differential regulating effect of cholesterol plus 25-hydroxycholesterolesterols can probably be explained by the stronger regulatory challenge elicited by the exogenous sterols. The sterols are added in ethanol, which allows them to enter the cytosol in high concentration bypassing the requirement for receptors that are saturated if LDL is added. Furthermore, 25-hydroxycholesterol is up to 100 times more potent than cholesterol itself, probably due to the higher water solubility. Ho et al also studied the suppression of LDL receptor activity in leukemic cells by LDL or sterols. They found that LDL or oxygenated sterols suppressed LDL receptor activity in AML cells and concluded that the enhanced LDL receptor activity in AML cells is not due to a loss of sensitivity to regulation by LDL cholesterol. However, they only studied the effects at one time point (67 hours) and no comparisons were made with normal mononuclear cells regarding the suppressing effect of sterols. It is noteworthy that the maximum LDL receptor RNA copy number reached in AML cells during in vitro incubations (approximately 8 copies/cell) was not found in cells immediately after isolation from blood. In contrast, the maximum HMG-CoA reductase RNA level (3 copies/cell) was found both in freshly isolated cells and during prolonged incubation in lipoprotein-deficient medium. This discrepancy indicates differential regulation and a greater reserve capacity for the LDL receptor at the RNA level in these cells.

Additional mechanisms could be responsible for the high LDL receptor or HMG-CoA reductase gene expression in leukemic cells. The observations of a low cholesterol content and a high endogenous cholesterol synthesis in AML cells, in the current study as well as in previous studies, indicate a cellular deficiency of cholesterol that could be a driving force for increased LDL receptor or HMG-CoA reductase gene expression. Increased cholesterol demand for membrane synthesis in rapidly proliferating cells is one mechanism that could lower the intracellular cholesterol concentration. Indeed, LDL receptor activity in cultured cells is
Table 3. Regulation of LDL Receptor and HMG-CoA Reductase Gene Expression by LDL or Mevinolin in Normal and Leukemic Cells

<table>
<thead>
<tr>
<th>Additions</th>
<th>Duration of Prior Degradation of 14C-LDL (ng/h/10⁶ cells)</th>
<th>RNA Activity RNA (copies/cell)</th>
<th>HMG-CoA Reductase Activity (µmol/min/mg)</th>
<th>RNA (copies/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subject</td>
<td>0</td>
<td>0.43</td>
<td>0.53</td>
<td>2.60</td>
</tr>
<tr>
<td>3</td>
<td>7.49</td>
<td>1.85</td>
<td>23.7</td>
<td>2.54</td>
</tr>
<tr>
<td>LDL</td>
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<td>0.72</td>
<td>0.57</td>
<td>5.10</td>
</tr>
<tr>
<td>Healthy subject</td>
<td>0</td>
<td>0.67</td>
<td>0.49</td>
<td>5.8</td>
</tr>
<tr>
<td>3</td>
<td>9.72</td>
<td>5.71</td>
<td>35.2</td>
<td>1.57</td>
</tr>
<tr>
<td>LDL</td>
<td>3</td>
<td>1.28</td>
<td>0.84</td>
<td>7.5</td>
</tr>
<tr>
<td>AML</td>
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<td>1.74</td>
<td>1.24</td>
<td>17.4</td>
</tr>
<tr>
<td>3</td>
<td>14.8</td>
<td>8.16</td>
<td>43.1</td>
<td>1.87</td>
</tr>
<tr>
<td>LDL</td>
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<td>1.96</td>
<td>4.80</td>
<td>16.9</td>
</tr>
<tr>
<td>Mev.</td>
<td>3</td>
<td>64.8</td>
<td>22.7</td>
<td>1,650</td>
</tr>
<tr>
<td>AML</td>
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<td>2.00</td>
<td>1.10</td>
<td>45.1</td>
</tr>
<tr>
<td>3</td>
<td>28.5</td>
<td>5.02</td>
<td>439</td>
<td>2.02</td>
</tr>
<tr>
<td>LDL</td>
<td>3</td>
<td>5.53</td>
<td>2.07</td>
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</tr>
<tr>
<td>Mev.</td>
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<td>15.3</td>
<td>4143</td>
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<tr>
<td>ALL</td>
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<td>0.15</td>
<td>0.52</td>
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</tr>
<tr>
<td>3</td>
<td>0.28</td>
<td>1.29</td>
<td>148</td>
<td>3.00</td>
</tr>
<tr>
<td>LDL</td>
<td>3</td>
<td>0.13</td>
<td>1.00</td>
<td>57.2</td>
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</tbody>
</table>

LDL receptor and HMG-CoA reductase RNA levels and protein activities were determined in mononuclear blood cells from healthy individuals and patients with acute leukemia directly after isolation from blood (day 0) and after 3 days of culture (day 3) in medium containing 10% LPDS in the presence or absence of LDL (50 µg/mL) or mevinolin (1 µmol/L).

highly dependent on the growth rate.31 Another possibility is that leukemic cells require more cholesterol because of rapid membrane turnover. Low intracellular cholesterol levels as a mechanism for stimulation of LDL receptor or HMG-CoA reductase gene expression are supported by the effects of prolonged incubations of cells in medium lacking lipoproteins. The larger increases in protein activities relative to RNA mimic the situation in freshly isolated leukemic cells with increased LDL receptor activity and elevated ratios of protein activity to RNA level compared with normal cells.

The altered cholesterol homeostasis in leukemic cells could be of potential interest for selective treatment of acute leukemia. Previous studies have shown that cytotoxic agents can be incorporated into LDL and delivered to tumor cells both in vitro and in vivo.32 During such LDL-targeted chemotherapy, there would be a potential risk for toxicity in organs with high LDL uptake, such as the adrenals and the liver. The postmortem content of 14C-sucrose-LDL in the adrenals (per gram of tissue) was in fact about 7 times higher than in the liver and 9 times higher than in the leukemic bone marrow in 1 patient with AML.33 However, animal studies indicate that it is possible to down regulate the LDL uptake in the adrenals and the liver by pretreatment with steroids and bile acids without affecting the uptake by the tumor.33 Patients whose leukemic cells express high LDL receptor activity should hence be considered for targeted drug therapy with LDL-bound drugs and patients whose leukemic cells express a high HMG-CoA reductase activity should be considered for therapy with competitive HMG-CoA reductase inhibitors. Indeed, antitumoral effects of HMG-CoA reductase inhibitors have been demonstrated in animal tumor models in vivo.34,35

ACKNOWLEDGMENT

We thank Eva Wärdell for excellent technical assistance.

Table 4. Regulation of LDL Receptor Gene Expression in Normal and Leukemic Cells by LDL or Sterols

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Degradation of 14C-LDL (ng/h/10⁶ cells)</th>
<th>LDL Receptor RNA (copies/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 15 15 15 15</td>
<td>0 2 2 2 2 15 15 15 15</td>
<td>1.2/24 1.2/24 1.2/24 1.2/24 1.2/24 1.2/24 1.2/24 1.2/24</td>
</tr>
<tr>
<td>LDL</td>
<td>0.29 1.28 0.54 0.36 0.00</td>
<td>0.42 0.50 0.47 0.34 0.85 0.34 0.68 0.18</td>
</tr>
<tr>
<td>25OH-Chol/Chol</td>
<td>0.29 1.28 0.54 0.36 0.00</td>
<td>0.42 0.50 0.47 0.34 0.85 0.34 0.68 0.18</td>
</tr>
<tr>
<td>Healthy subject</td>
<td>0.70 1.97 0.53 0.54 0.18</td>
<td>0.62 0.99 0.77 0.78 0.36 1.53 0.46 0.35 0.18</td>
</tr>
<tr>
<td>AML</td>
<td>6.74 50.9 8.35 7.30 5.69</td>
<td>11.8 7.00 6.28 6.13 3.39 5.97 2.07 1.94 0.97</td>
</tr>
</tbody>
</table>

LDL receptor RNA levels and protein activity were determined in mononuclear blood cells from healthy individuals and 1 patient with AML directly after isolation from blood, and after 2 and 15 hours of incubation in medium containing 10% LPDS and the indicated additions. The concentrations of LDL and a mixture of 25-hydroxycholesterol + cholesterol (25OH-Chol/Chol) are given in micrograms per milliliter. The mixture of sterols was added in ethanol (6 µL ethanol/mL medium).
REFERENCES

Multilevel regulation of low-density lipoprotein receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase gene expression in normal and leukemic cells

S Vitols, S Norgren, G Juliusson, L Tatidis and H Luthman