Decreased feedback regulation of low density lipoprotein receptor activity by sterols in leukemic cells from patients with acute myelogenous leukemia

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Abstract Leukemic cells from patients with acute myelogenous leukemia (AML) have higher low density lipoprotein (LDL) receptor activity than normal white blood and bone marrow cells. The underlying mechanism behind this is unclear. We studied the inhibitory effect of sterols on induction of LDL-receptor activity in leukemic cells from 27 patients with AML and in white blood cells from 13 healthy individuals. The high affinity degradation rate of ^{125}I-labeled LDL was determined in mononuclear blood cells directly after isolation from blood and after incubation for 2 days in medium with 10% lipoprotein-deficient serum with or without various concentrations of 25-hydroxycholesterol + cholesterol. The median sterol concentration for 50% inhibition (IC_{50}) of induction was more than five times higher for leukemic cells than for normal mononuclear cells. At the highest sterol concentration (0.400 μg/mL 25-hydroxycholesterol + 8 μg/mL cholesterol), the LDL-receptor activity was abolished in cells from all healthy individuals while the induction of LDL-receptor activity in cells from three AML patients was unaffected. The LDL-receptor activity of leukemic cells, directly after isolation from blood, correlated with IC_{50} values ($r = 0.53$, $P = 0.007$) and WBC counts ($r = 0.72$, $P = 0.0001$) but not with cellular cholesterol levels. The results demonstrate decreased feedback regulation of LDL-receptor activity by sterols in AML cells and support the conclusion that elevated LDL-receptor activity is associated with sterol resistance and cell proliferation. The findings are of potential interest for diagnosis and specific treatment of leukemia.—**Tatidis, L., A. Gruber, and S. Vitols.** Decreased feedback regulation of low density lipoprotein receptor activity by sterols in leukemic cells from patients with acute myelogenous leukemia. *J. Lipid Res.* 1997. **38:** 2436–2445.

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Low density lipoprotein (LDL) is the major cholesterol-carrying lipoprotein in human plasma. Cells use cholesterol for membrane synthesis and as a precursor for steroid hormones and bile acids. The cholesterol demands are met either by receptor-mediated endocytosis of LDL from plasma or by de novo synthesis, of which the rate-limiting step is catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. After binding to the receptor and endocytosis, the LDL particle is hydrolyzed in lysosomes that yield unesterified cholesterol for the cells use (1).

Both pathways are regulated coordinately by the intracellular cholesterol level (2–4). Sterol suppressive transcription of the LDL-receptor gene is controlled by a 10 base pair sequence in the 5′ flanking region, designated sterol regulatory element one (SRE-1) (3,5,6). Recently sterol regulatory element biding proteins (SREBPs) have been cloned and characterized (7, 8). When the intracellular concentration of cholesterol is low, SREBPs bind to SRE-1, initiate transcription, and LDL-receptor activity is increased. When cellular sterol levels are high, transcription is reduced and receptor activity decreases (9). Transcription of the HMG-CoA reductase gene is also regulated by SREBPs, although full transcriptional activation is thought to require another factor as well (10).

Previous studies on mononuclear blood cells isolated from patients with acute myelogenous leukemia (AML) have shown elevated LDL-receptor activity compared to normal white blood cells and nucleated bone marrow cells (11, 12). The elevated LDL-receptor activity in leukemic cells from patients with AML has also been confirmed by an in vivo study using [^{14}C]sucrose-LDL which was given intravenously (13). Hypocholester-

Abbreviations: LDL, low density lipoprotein; AML, acute myelogenous leukemia; LPDS, lipoprotein-deficient serum; PBS, phosphate-buffered saline.

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olemia, which is common in AML, probably is a consequence of the elevated LDL-receptor activity in leukemic cells (14). However, the mechanism that is responsible for elevated LDL-receptor activity is not known. Recent data from our laboratory on leukemic and normal mononuclear blood cells suggest, in addition to transcriptional regulation of the LDL-receptor, other regulatory levels also (15). We also observed that sterols (25-hydroxycholesterol + cholesterol) could not suppress the induction of LDL-receptor activity in AML cells from a patient with high LDL-receptor activity as effectively as in normal cells, indicating that the elevated LDL-receptor activity might be due to a decreased feedback regulation by sterols (15). In this study we therefore systematically compared regulation of LDL-receptor activity by sterols in leukemic cells from patients with AML with that of white blood cells from healthy individuals.

METHODS

Patients with leukemia and normal subjects

White blood cells were analyzed from 27 patients (14 men and 13 women) with AML; their mean age was 62 years (range 29 to 90). The healthy subjects, who were blood donors at the Karolinska hospital, consisted of 13 individuals (8 men and 5 women); their mean age was 41 years (range 21 to 66). Based on morphology, cytochemistry, and immunphenotype the leukemias were subtyped according to the French-American-British (FAB) classification (**Table 1**); (acute myeloblastic leukemia with no maturation (M0), minimal maturation (M1), some maturation (M2), acute promyelocytic leukemia (M3), acute myelomonocytic leukemia (M4), acute monoblastic leukemia without (M5A), and with (M5B) maturation (16)). Approval for the study was obtained from the local ethics committee.

Solutions

Mevinolin (provided by Dr. A. W. Alberts, Merck Sharp & Dome, Rahway, NJ) in the lactone form was converted to the sodium salt by heating at 50°C for 1 h in 0.1 mol/L NaOH, after which the solution was neutralized with 1 mol/L HCl.

Lipoproteins

LDL (d 1.020–1.063 gm/mL) and human lipoprotein-deficient serum (LPDS; d > 1.215 g/mL) were isolated from serum of healthy blood donors by sequential ultracentrifugation (17). The purity of LDL and LPDS preparations was examined by agarose gel electrophoresis, and the absence of cholesterol in LPDS was confirmed by enzymatic cholesterol analysis (Merck, Darmstadt, FRG). ^{125}I-labeled LDL (sp act 130–375 cpm/ng protein) was prepared as described by Langer, Strober and Levy (18). Less than 1% of the radioactivity in the ^{125}I-labeled LDL preparations was present as free iodide. The concentrations of LDL refer to protein and were determined by the method of Lowry et al. (19) with bovine serum albumin as standard.

Isolation of cells and determination of LDL-receptor activity

Mononuclear blood cells were isolated at 4°C by centrifugation on Lymphoprep (d 1.077 g/mL) (Nycomed Pharma AS, Oslo, Norway) (20), and washed three times with phosphate-buffered saline (PBS; 140 mmol/L NaCl, 2.7 mmol/L KCl, 9.5 mmol/L Na_2HPO_4, and 9.5 mmol/L KH_2PO_4, pH 7.4). Granulocytes were separated from blood by sedimentation in PBS supplemented with 2% (w/v) dextran T500 (Pharmacia, Uppsala, Sweden) and 100 IU/mL of heparin for 30 min at 4°C (20), followed by centrifugation on Lymphoprep as above. The resulting pellet containing granulocytes and erythrocytes was washed in PBS. Remaining erythrocytes were removed by hypotonic lysis followed by an additional wash with PBS.

The high affinity (receptor-mediated) degradation rate of ^{125}I-labeled LDL was used as a measure of LDL-receptor activity (11, 12). In brief, 3×10^6 cells were incubated in 35 × 10 mm tissue culture dishes (Costar Corporation, Cambridge, MA) at 37°C in 1 mL of RPMI 1640 medium supplemented with 10% LPDS and antibiotics (100 IU penicillin + 100 μg streptomycin/mL) with 25 μg ^{125}I-labeled LDL in the absence or presence of 500 μg unlabeled LDL. After 4 h incubation, the formation of noniodide TCA-soluble radioactivity in the medium was determined. The high-affinity degradation rate of ^{125}I-labeled LDL was calculated by subtracting the degradation of ^{125}I-labeled LDL in the presence of excess unlabeled LDL (nonspecific degradation) from the degradation of ^{125}I-labeled LDL in the absence of unlabeled LDL (total degradation).

Regulation of LDL-receptor activity by sterols

In order to study the inhibitory effect of sterols on induction of LDL-receptor activity, 3×10^6 cells were incubated in 35 × 10 mm tissue culture dishes in 1 mL of 10% LPDS medium and antibiotics with or without different concentrations of a mixture of 25-hydroxycholesterol (Steraloids, Inc., Wilton, NH) and cholesterol (Sigma Chemical Co., St. Louis, MO) in a ratio of 1:20 added in 2 μL ethanol. In one experiment, mevinolin (final concentration 1 μmol/L) was also added to the dishes. After 2 days of incubation at 37°C, the high af-

TABLE 1. Clinical characteristics, degradation values, and cellular and plasma cholesterol levels for 27 patients with AML and 13 healthy individuals

No.	Sex	Age	Diagnosis FAB	WBC	High Affinity Degradation of ^{125}I-labeled LDL		Plasma Cholesterol		Cellular Cholesterol
					Day 0[a]	Day 2[b]	Total	LDL	
		yr		*$\times 10^9$/L*	*ng/h/10^6 cells*		*mmol/L*		*μg/mg protein*
1	M	74	MO	90	0.33	4.08	3.50	2.35	12.66
2	F	67	M1	88	0.81	11.77	4.30	2.81	12.76
3	F	63	M1	50	1.01	6.55	3.47	1.39	14.17
4	M	69	M1	40	0.44	0.49	4.62	2.72	10.03
5	F	40	M2	95	1.22	57.65	3.39	2.22	9.23
6	F	58	M2	90	1.35	8.34	5.09	3.19	12.16
7	F	73	M2	79	1.13	19.81	5.11	3.38	8.67
8	F	71	M2	55	1.39	20.99	2.68	1.37	11.38
9	M	38	M2	50	0.91	6.71	2.82	1.24	nd
10	F	88	M2	40	2.44	26.75	4.00	2.60	8.92
11	F	75	M2	30	2.23	22.98	3.83	2.52	17.46
12	F	52	M2	29	0.80	5.10	3.65	3.02	7.7
13	M	88	M2	26	1.09	1.75	4.73	2.39	11.00
14	M	82	M2	18	2.11	18.57	2.45	1.71	8.03
15	F	66	M4	283	7.20	40.62	3.41	2.10	11.44
16	M	40	M4	231	2.74	24.48	4.75	2.33	17.83
17	M	43	M4	200	2.31	35.19	2.64	0.99	20.18
18	M	33	M4	120	1.51	24.22	2.17	0.93	14.31
19	M	80	M4	22	0.46	58.37	3.82	2.67	nd
20	F	29	M5A	100	1.87	7.08	5.23	3.20	13.34
21	M	53	M5A	100	1.32	18.52	3.41	1.51	12.95
22	M	57	M5A	90	4.94	46.37	2.92	1.86	12.56
23	F	58	M5B	45	3.73	18.35	3.31	1.78	14.12
24	M	88	M5	59	3.66	1.88	4.03	1.88	12.41
25	M	55	AML[c]	350	8.70	22.86	2.15	0.99	9.36
26	F	70	AML[c]	115	1.76	9.86	5.22	3.67	11.17
27	M	90	AML[c]	100	0.14	2.51	nd	nd	10.21
28	M	52	m	nd	0.42	7.75	3.68	2.94	9.54
29	M	62	m	nd	0.48	6.22	5.72	4.77	15.60
30	M	45	m	nd	0.40	2.89	6.65	4.83	22.30
31	M	39	m	nd	1.05	8.74	4.32	2.34	19.64
32	M	38	m	nd	0.46	5.49	5.52	4.20	15.63
33	F	45	m/g	nd	0.48/0.50	3.9/2.55	5.21	3.20	17.08/11.22
34	M	32	m	nd	0.51	25.03	5.58	3.38	15.18
35	M	22	m	nd	0.57	1.89	3.81	1.27	nd
36	F	21	m	nd	0.92	4.37	5.18	2.55	21.91
37	M	46	m	nd	0.41	7.41	6.64	4.38	17.71
38	F	66	m/g	nd	0.57/0.64	5.57/2.70	5.87	3.70	nd/nd
39	F	38	m	nd	0.34	2.33	5.62	3.06	nd
40	F	25	g	nd	0.39	3.50	2.56	0.94	10.63

Abbreviations: m, normal mononuclear blood cells; g, granulocytes; nd, not determined.
[a]Degradation directly after isolation from blood.
[b]Degradation after 2 days of incubation in medium with 10% LPDS.
[c]Not subclassified according to FAB.

finity degradation of ^{125}I-labeled LDL was determined as described above. The sterol concentration for 50% reduction of day 2 activity (IC_{50}), which was used as an estimate of sterol sensitivity, was determined graphically for each individual. Cells from a healthy subject were also preincubated at a cell concentration of 3×10^6 cells/mL in 24 mL 10% LPDS medium in 75 cm^2 tissue culture flasks (Costar Corporation, Cambridge, MA) for 24 h in the presence of either 50 μg/mL of LDL, 1 μmol/L mevinolin, or without any addition. The cells were then centrifuged and washed two times with PBS and incubated with or without sterols as above.

The inhibitory effect of LDL on induction of LDL-receptor activity was also studied. Approximately 3×10^6 cells/mL were incubated in 25 cm^2 tissue culture flasks (Costar Corporation, Cambridge, MA) in 8 mL 1640 RPMI medium supplemented with 10% LPDS and antibiotics with or without LDL in various concentrations. After 2 days of incubation the cells were centrifuged and washed two times with PBS and the high affinity degradation of ^{125}I-labeled LDL was determined as described above.

Recounting after incubation for 2 days showed that no significant change in cell number occurred either among normal cells or leukemic cells.

Determination of cholesterol in cells and plasma

For analysis of cellular cholesterol content, a combination of an enzymatic method and high pressure liquid chromatography was used with some modifications (21). Approximately 50–100 × 10^6 cells were dissolved in 5–10 mL 0.5% (w/v) sodium cholate (Sigma Chemical Co., St. Louis, MO) and sonicated for 30 sec using a Vibra cell VC50T sonifier (Sonics & Materials Inc., Danbury, CT). Two 100-μL aliquots of the solution were taken for measurement of free and total cholesterol. The 100-μL aliquots were supplemented with 10 μL of a reaction mixture containing 500 mM $MgCl_2$, 500 mM Tris-HCl (pH 7,4), 10 mM dithiothreitol, and 1% (v/v) Triton X-100. Thereafter cholesterol oxidase (Boeringer Mannheim, GmbH, Germany) or a mixture of cholesterol oxidase and cholesterol esterase (Sigma Chemical Co., St. Louis, MO) was added in 10 μL of 0.5% sodium cholate (final concentration 0.4 U/mL) for determination of free cholesterol and total cholesterol, respectively. The tubes were incubated at 37°C for 30 min and then 380 μL methanol-ethanol 1:1 was added to stop the reaction. Samples were left for 30 min on ice and then centrifuged for 30 min at 1500 *g*. Fifty microliters of the supernatant was injected into the high pressure liquid chromatography system. Samples were separated on a Beckman Ultrasphere ODS 5μ 4.6 mm × 25 cm C-18 column (Beckman Instruments, Inc., Fullerton, CA) with an RCSS CN C-18 Guard-Pak precolumn insert (Waters Corporation, Milford, MA). Methanol–acetic acid 99:1 was used as eluent at a flow rate of 1.8 mL/min, and absorbance was determined at 240 nm. The cellular cholesterol content was expressed as μg/mg protein, which was determined by the method of Lowry et al. (19).

Plasma total and LDL cholesterol were determined by the CHOD-PAP Mercotest kit (Merck, Darmstadt, FRG).

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 <0.05 were considered significant.

RESULTS

The mean and SD high affinity degradation rates of ^{125}I-labeled LDL directly after isolation from blood were 0.55 ± 0.21 and 2.13 ± 2.03 ng/h per 10^6 cells, for mononuclear cells from 12 healthy subjects and leukemic cells from 27 patients with AML, respectively ($P = 0.0004$). After incubation for 2 days in medium with 10% LPDS, the degradation rate increased in both leukemic and normal cells with considerable interindividual variation (Table 1, **Fig. 1**). The mean day 2 ^{125}I-labeled LDL degradation rates were 6.8 ± 6.14 and 19.33 ± 16.40 ng/h per 10^6 cells for normal mononuclear and leukemic cells, respectively ($P = 0.0014$).

The increase in degradation rate in mononuclear cells from all healthy subjects was effectively suppressed in the presence of sterols (Fig. 1 and **Fig. 2**) with low interindividual variation (median IC_{50} value of 25-hydroxycholesterol 0.029 μg/mL; range, 0.015–0.045). In contrast, the leukemic cells responded less and with a large interindividual variation (median IC_{50} value of 25-hydroxycholesterol 0.155 μg/mL; range 0.045 to >0.400 μg/mL) to the inhibitory action of sterols (Fig. 1 and Fig. 2). Leukemic cells from three subjects (nos. 8, 10, and 15) were totally unresponsive to sterols. There was a correlation between the IC_{50} values for AML cells and the degradation rates directly after isolation from blood ($r = 0.53$, $P = 0.007$, $n = 24$, **Fig. 3**). Resistance to the inhibitory effect of sterols on induction of LDL-receptor activity in AML cells was also observed when LDL was used as the source of cholesterol (**Fig. 4**).

Granulocytes from healthy subjects behaved similar to normal mononuclear cells with regard to the ^{125}I-labeled LDL degradation rate directly after isolation from blood and after induction in the absence or presence of sterols (Table 1 and Figs. 1 and 2).

In order to investigate whether increased cellular cholesterol requirements could explain the altered response to sterols, cells were incubated under different conditions during or prior to the sterol sensitivity test. The addition of the HMG-CoA reductase inhibitor mevinolin, during the incubation with sterols, did not influence the response to sterols in normal mononuclear cells as compared with the incubation without mevinolin, in spite of the fact that the presence of mevinolin increased the degradation value on day 2 from 3.9 to 6.3 ng/h per 10^6 cells (not shown). Likewise, when normal mononuclear cells were preincubated for 24 h with either LDL (50 μg/mL), mevinolin (1 μM), or without any addition in 10% LPDS medium, no significant effect on the subsequent response to sterols was observed although the degradation rates differed severalfold after the preincubations, and were 0.3, 1.4, and 1.0 ng/h per 10^6 cells, respectively (not shown).

The cellular cholesterol content (Table 1) was significantly lower in leukemic cells than in normal mononuclear cells. The mean and SD cellular free cholesterol contents were 17.17 ± 3.91 μg/mg protein ($n = 9$) and 12.16 ± 3.08 μg/mg protein ($n = 25$) for normal and leukemic cells, respectively ($P = 0.005$). The cellular free cholesterol content was >80% of the total (free + esterified) cholesterol in all cells tested.

No correlation was observed between the ^{125}I-labeled

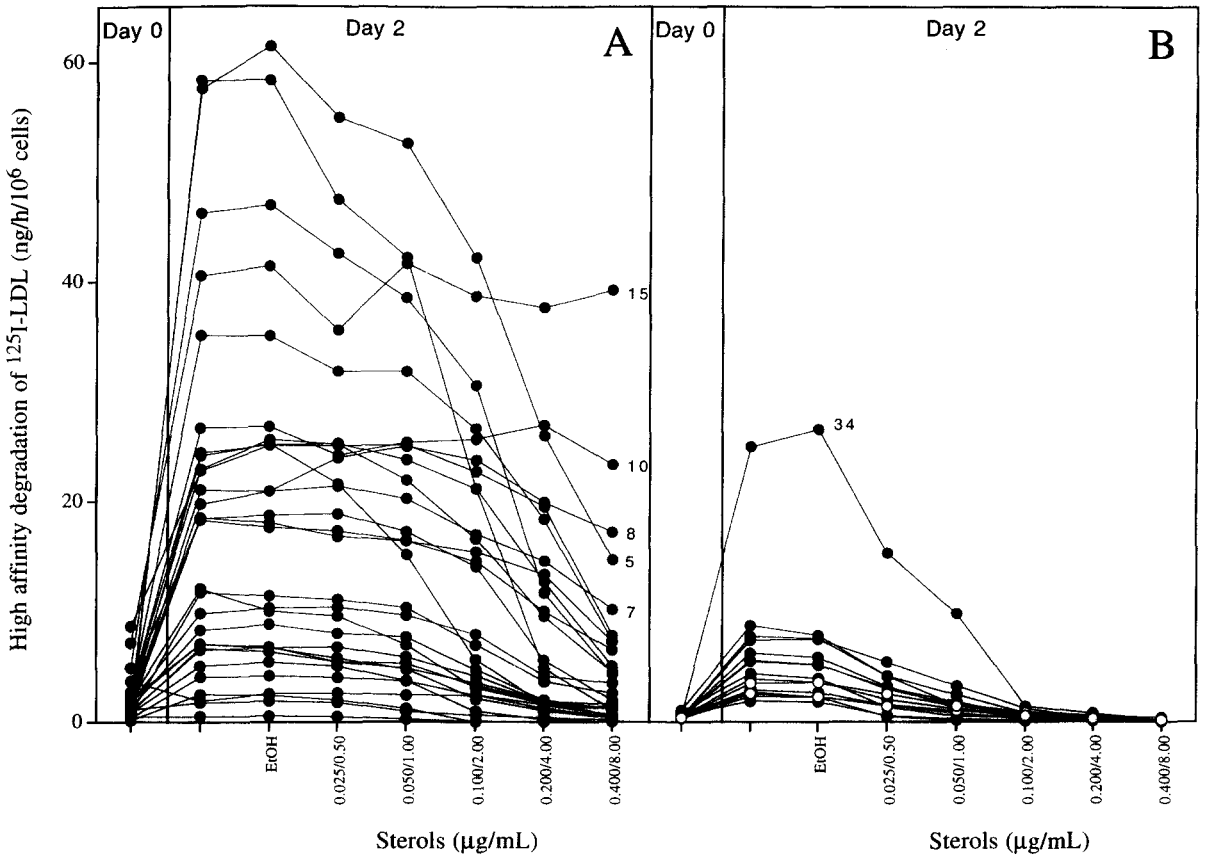

Fig. 1. High-affinity degradation rate of ^{125}I-labeled LDL in mononuclear blood cells from 27 patients with AML (A) and 12 healthy blood donors (B) directly after isolation (day 0) and after 2 days (day 2) of incubation in medium containing 10% LPDS with or without various concentrations of 25-hydroxycholesterol and cholesterol (added as a mixture in a ratio of 1:20). The results from granulocytes (open circles) isolated from three healthy blood donors are also given in B. The numbers on the X-axis denote concentrations of 25-hydroxycholesterol and cholesterol in μg/mL. Each data point is the mean of triplicate incubations. The numbers inside the graph refer to subjects in Table 1.

LDL degradation rate and the free cholesterol content in leukemic ($r = 0.018$, $P = 0.933$) or normal cells ($r = 0.459$, $P = 0.214$) directly after isolation from blood. Likewise, no significant correlation was observed between the degradation rate and cellular free cholesterol content when mononuclear cells from patients with leukemia and healthy subjects were analyzed together ($r = 0.164$, $P = 0.353$). In contrast, a strong correlation was observed between the degradation rate in freshly isolated leukemic cells and the WBC count in patients with AML ($r = 0.72$, $P = 0.0001$).

DISCUSSION

In the present study we demonstrated that mononuclear blood cells from patients with AML have a reduced sensitivity towards the suppressive effect of sterols on LDL-receptor activity, compared to white blood cells from healthy individuals. Of particular interest is the significant correlation obtained between the high affinity degradation rate of ^{125}I-labeled LDL in AML cells directly after isolation from blood and resistance to down-regulation of LDL-receptor activity by sterols (IC_{50} values). This finding indicates that the elevated LDL-receptor activity in AML cells could be a result of decreased feedback regulation by cholesterol or some other regulatory sterol.

It could be speculated that the elevated LDL-receptor activity in freshly isolated AML cells is a compensatory response to mechanisms that lower the cellular cholesterol content, such as increased cholesterol demands for membrane synthesis due to rapid cell proliferation and/or a high membrane turnover rate or "leakage" of cholesterol or some of its metabolites, which subsequently leads to sterol resistance. This hypothesis is supported by the findings in the present

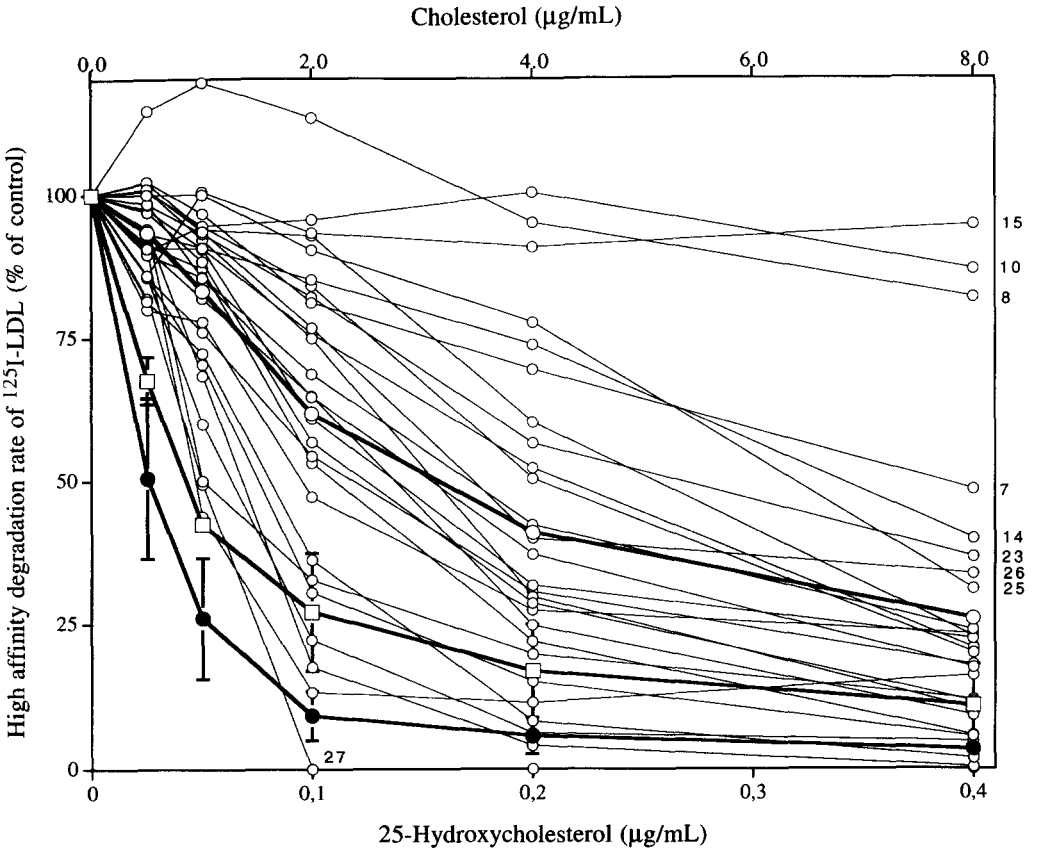

Fig. 2. High-affinity degradation rate of ^{125}I-labeled LDL in mononuclear blood cells from 27 patients with AML (open circles) after 2 days of incubation in medium containing 10% LPDS with or without various concentrations of 25-hydroxycholesterol and cholesterol (added as a mixture in a ratio of 1:20), expressed as percent of control without sterols. Each data point is the mean of triplicate incubations. The mean for all patients with leukemia (thick line, open circles) as well as the means and SD for mononuclear blood cells from 12 healthy blood donors (thick line, filled circles) and granulocytes from three healthy blood donors (thick line, squares) are also given. The numbers refer to subjects in Table 1.

study as well as in other studies of a lower cholesterol content (22–24) and elevated cholesterol synthesis rates (15, 25) in AML cells compared with normal cells. However, we found no correlation between the cellular free cholesterol content and the high affinity ^{125}I-labeled LDL degradation rate in AML cells directly after isolation from blood, indicating that this is not the mechanism. We cannot of course rule out the possibility that there might still be a lower concentration of cholesterol or its derivatives in the regulatory compartment but it is unlikely that this would have been detected against the background of cholesterol in the membranes of the whole cell extract. If a low cholesterol content in AML cells were the major force for an elevated LDL-receptor activity, this would, however, be reversed by high concentrations of 25-hydroxycholesterol/cholesterol and subsequently cause a down-regulation of LDL-receptor activity. This was not the case in AML cells in contrast to normal cells where the highest concentrations of sterols completely abolished LDL–receptor activity. Also, incubation of normal cells under conditions that caused increased cellular cholesterol demands did not change the response to sterols, clearly demonstrating that sterol resistance is not solely a consequence of increased cellular cholesterol requirements.

In mutant cell lines, defective down-regulation of the LDL-receptor and of HMG-CoA reductase activities by sterols has been described. A sterol resistant Chinese hamster ovary (CHO) cell line showed loss of sterol-mediated repression in the expression of the LDL-receptor, HMG-CoA reductase, and HMG-CoA synthase genes, as well as the activities of the corresponding proteins (26). Later it was shown that the sterol-resistant CHO cells produced a truncated active form of SREBP-2, which initiates transcription of the LDL-receptor and HMG-CoA synthase genes independently of the sterol-regulated proteolytic step, normally activating SREBP in the absence of sterols, thereby eliminating sterol sen-

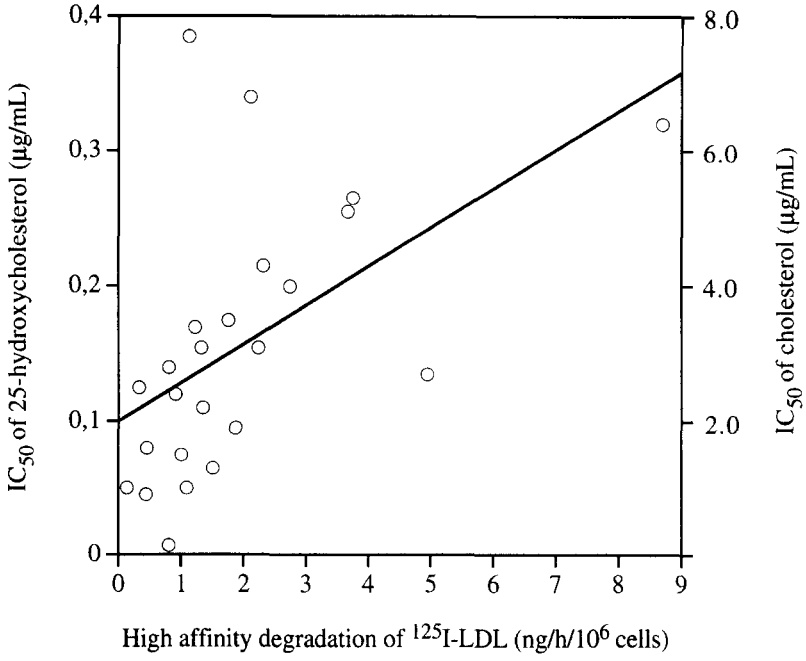

Fig. 3. Relation between the high-affinity degradation rate of ^{125}I-labeled LDL in mononuclear blood cells from 24 patients with AML directly after isolation and the concentrations of 25–hydroxycholesterol and cholesterol (added as a mixture in a ratio of 1:20) for 50% reduction (IC_{50}) of the degradation rate on day 2. Each data point represents the mean of triplicate incubations.

sitivity (27). In another acyl-CoA:cholesterol acyltransferase (ACAT) mutant CHO cell line, it was found that activation of SREBPs was not suppressed by 25-hydroxycholesterol resulting in a sterol-resistant phenotype (28, 29). Also, in tumor-transformed fibroblasts, a defective down-regulation of HMG-CoA reductase activity by LDL-cholesterol was associated with a decreased formation of 27-hydroxycholesterol and other cholesterol derivatives that are potent regulators of HMG-CoA reductase activity (30).

It could be argued that decreased feedback regulation by sterols cannot be the only explanation to our results. If, during the prolonged incubation in 10% LPDS, LDL-receptor activity increases due to removal of sterol regulation, why then does not incubation with sterols or LDL reduce the activity to the freshly isolated level? Several explanations in addition to a resistance to sterol regulation could be suggested. First, the LDL-receptor activity of the cells, directly after isolation, is low for some artefactual reason (e.g., cell damage) and the incubation in 10% LPDS in some way restores the metabolic activity of the cells. This explanation is unlikely because if cell damage takes place during isolation, this should also apply to normal mononuclear cells. But here incubations with sterols in the highest concentrations inhibited the activity to the freshly isolated level or below. Moreover, previous studies have demonstrated, with a few exceptions, minor intrapatient variation in LDL-receptor activity of freshly isolated cells during consecutive samplings (14). Also, accumulation of intravenously administered [^{14}C]sucrose-LDL in AML cells showed a strong correlation with the in vitro determined LDL-receptor activity, supporting the conclusion that the in vitro determined 'degradation value' is 'correct' (13). Second, the high LDL-receptor activity is due to replication of the cells during the prolonged incubations in 10% LPDS which does not occur in freshly isolated cells. This was, however, not the case as the cell number did not increase significantly during the incubation in 10% LPDS. The third and most likely explanation is that the LDL-receptor activity in freshly isolated cells as well as the activity after incubation for 2 days in 10% LPDS are regulated not only by sterols but also by other non-sterol-mediated mechanisms.

LDL-receptor expression has been shown to be influenced by non-sterol mediated mechanisms. Mitogenic stimulation of various human cells leads to increased LDL-receptor mRNA expression and increased receptor activity (31–35). In a human leukemic T cell line, it was shown that mitogen-induced increase of LDL-receptor gene expression was not regulated by sterols (36). It may, therefore, be speculated that mitogenic stimulation of AML cells in vivo leads to cell growth and induces sterol-independent regulation of the LDL-receptor activity. The positive correlation obtained between the WBC count and the LDL-receptor activity in cells from AML patients directly after isolation from blood also supports the conclusion that the mechanism behind elevated LDL-receptor activity is associated with growth stimulation of the cells. Indeed, cell proliferation has been shown to stimulate LDL-receptor activity in cultured cells (37).

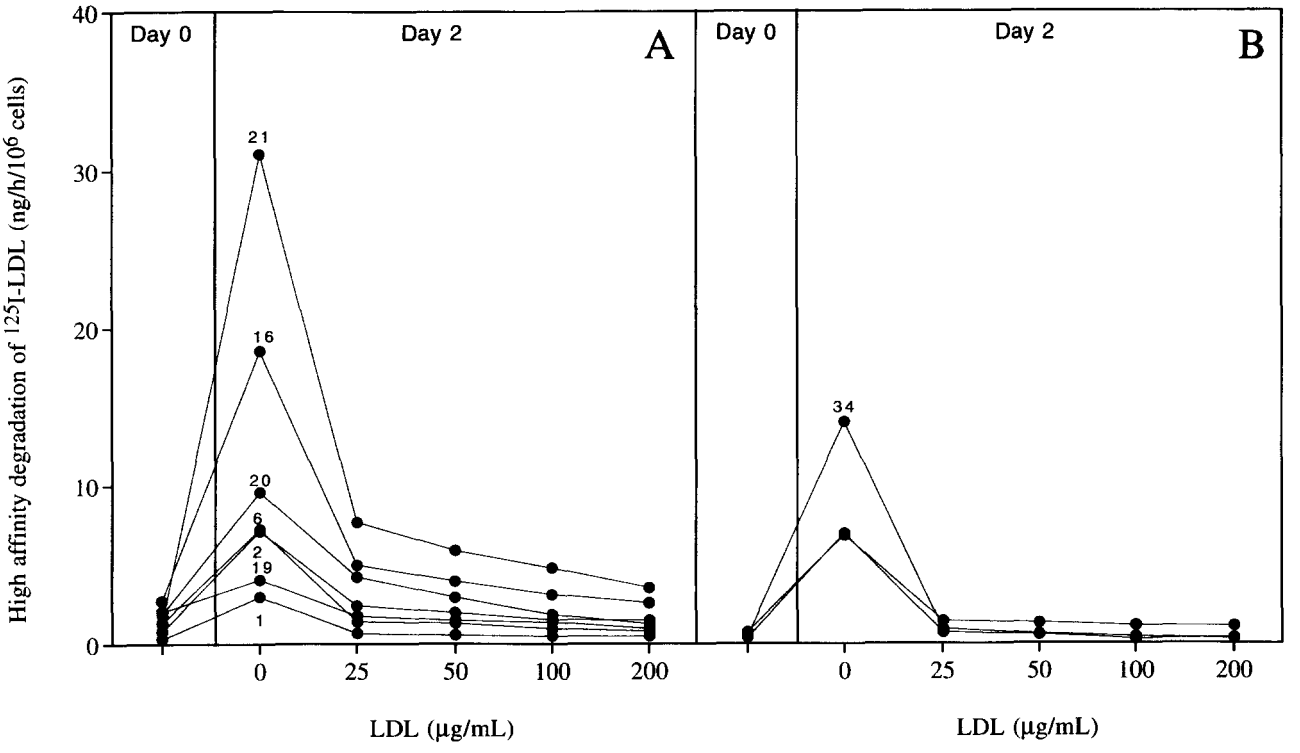

Fig. 4. High-affinity degradation rate of ^{125}I-labeled LDL in freshly isolated mononuclear blood cells from patients with AML (A) and healthy blood donors (B) directly after isolation (day 0) and after 2 days (day 2) of incubation in medium containing 10% LPDS alone or with the indicated concentrations of LDL in. Each data point represents the mean of triplicate incubations. The numbers inside the graph refer to subjects in Table 1.

Several of the mechanistic explanations discussed here could be responsible for the elevated LDL-receptor activity in AML cells. The fact that mitogenic stimulation leads to elevated LDL-receptor activity and sterol-independent regulation in cultured cells, which resembles the sterol resistance found in AML cells in the current study, indicates that mitogenic stimulation of AML cells in vivo could be the primary mechanism. It is possible that other mechanisms, such as leakage of cholesterol or its metabolites from cells or altered function of SREBPs, act simultaneously in some patients. The great patient-to-patient variation in degradation rates and response to sterols supports the idea that multiple mechanisms could be involved. A part of this variation in LDL-receptor activity in freshly isolated cells is, however, explained by the FAB type of the leukemia as cells from patients with leukemias with monocytic differentiation (FAB M4 and M5) exhibited the highest degradation rates (12).

This study presents evidence that leukemic cells from patients with AML have altered cholesterol regulatory mechanisms. Such aberrations could facilitate neoplastic cell growth by increasing the supply of cholesterol. The decreased sensitivity to sterols found in AML cells described in this study could be beneficial for the concept of selective treatment of leukemia by LDL-targeted chemotherapy (38). As hypocholesterolemia is a common finding in AML, leukemic cells with high LDL-receptor activity would continue to accumulate cytotoxic agents incorporated into LDL while normal cells would down-regulate their LDL receptor activity and drug uptake after measures that raise the cholesterol level in plasma. Characterization of the factors that are involved in regulation of LDL-receptor and HMG-CoA reductase activities in normal cells but are altered in leukemic cells could offer new targets for cancer therapy.

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