Low-Density Lipoprotein (LDL) Receptor Activity in Human Acute Myelogenous Leukemia Cells

By Y. K. Ho, R. Graham Smith, Michael S. Brown, and Joseph L. Goldstein

The rate of receptor-mediated uptake and degradation of 125I-labeled low-density lipoprotein (LDL) was 3–100-fold higher in blood mononuclear cells from 7 patients with acute myelogenous leukemia (AML) as compared with cells from 18 healthy subjects and 21 patients with acute and chronic lymphocytic leukemias, infectious mononucleosis, and nonhematologic malignancies. The rate of cholesterol synthesis from 14C-acetate was also higher (2–30-fold) in the AML cells. The total rate of input of cholesterol (i.e., the sum of LDL-derived and endogenously synthesized cholesterol) in the AML cells was nine fold higher on the average than in the mononuclear cells from normal subjects. In both the normal and AML cells, more than 90% of the cholesterol input was derived from receptor-mediated degradation of LDL and less than 10% from cholesterol synthesized within the cell. Despite the higher input of cholesterol in the AML cells, the cellular content of cholesterol, as measured by cholesterol:protein ratio, was 50% lower than in normal mononuclear cells. These data indicate that the turnover of cellular cholesterol is more rapid in AML cells than in normal mononuclear cells. This enhanced turnover might be due to a more rapid rate of utilization of cholesterol for cellular growth or to a more rapid efflux of cellular cholesterol. The resultant depletion of cellular cholesterol elicits both a higher LDL receptor activity and a higher rate of cholesterol synthesis in these leukemic cells.

Human cells have been shown to derive cholesterol for membrane synthesis from two sources: they can synthesize cholesterol de novo from acetyl coenzyme A or they can obtain cholesterol from the receptor-mediated uptake and lysosomal degradation of plasma low-density lipoprotein (LDL). When grown in tissue culture medium containing lipoproteins, human cells such as fibroblasts, arterial smooth muscle cells, and lymphoblasts preferentially utilize the receptor system to obtain their cholesterol from LDL. Under these conditions, endogenous cholesterol synthesis is kept suppressed. Similar findings have recently been reported for human mononuclear cells freshly isolated from the bloodstream. Immediately after their isolation, both lymphocytes and monocytes synthesize cholesterol at a very low rate and preferentially use the receptor system to derive their cholesterol from plasma LDL.

Current evidence suggests that in certain types of acute leukemia the control mechanism for cellular cholesterol homeostasis may be disrupted. Freshly isolated leukemic cells from humans, mice, and guinea pigs have been reported to show an elevated rate of cholesterol synthesis when compared with normal...
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<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Gender</th>
<th>Diagnosis</th>
<th>White Blood Count (WBC)</th>
<th>Leukemia Type</th>
<th>Bone Marrow Findings</th>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>5/F</td>
<td>M</td>
<td>ALL</td>
<td>19,200</td>
<td>91% LB</td>
<td>PAS-block positive</td>
<td>Negative for T cell markers</td>
<td>None</td>
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<td>20</td>
<td>11/F</td>
<td>M</td>
<td>ALL</td>
<td>136,000</td>
<td>83% LB + ProL</td>
<td>PAS-block positive</td>
<td>TdT, 6.4 U/10^6 cells; negative for T cell markers</td>
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<td>21</td>
<td>16/M</td>
<td>M</td>
<td>ALL</td>
<td>230,000</td>
<td>92% LB</td>
<td>PAS-negative</td>
<td>TdT, 48 U/10^6 cells; negative for T cell markers</td>
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<tr>
<td>22</td>
<td>10/F</td>
<td>F</td>
<td>AML, myelomonocytic</td>
<td>12,300</td>
<td>52% Abn. M, 2% MB, 36% L, 6% P</td>
<td>PAS-finitely stippled SBB-positive</td>
<td>Auer rods; ↑ serum lysozyme</td>
<td>None</td>
</tr>
<tr>
<td>23</td>
<td>26/M</td>
<td>M</td>
<td>CML, blast crisis</td>
<td>79,600</td>
<td>80% MB, 5% ProM</td>
<td>SBB-positive</td>
<td>None</td>
<td>TRAMPCOL, 8–12 days prior to study</td>
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<td>24</td>
<td>27/M</td>
<td>M</td>
<td>AML, myelomonocytic</td>
<td>17,800</td>
<td>74% Abn. M, 7% MB</td>
<td>10% promyelocytes in marrow</td>
<td>None</td>
<td>5-Azacytidine</td>
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<tr>
<td>25</td>
<td>38/F</td>
<td>F</td>
<td>AML, myelomonocytic</td>
<td>152,000</td>
<td>19% Abn. M, 19% MB, 15% MC + MMC, 39% P, 6% L</td>
<td>SBB-positive</td>
<td>Marked gingival hyperplasia</td>
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<td>26</td>
<td>40/F</td>
<td>F</td>
<td>AML, myelomonocytic</td>
<td>48,800</td>
<td>45% MB, 5% MC, 10% B, 10% M, 13% L, 9% P</td>
<td>SBB-positive</td>
<td>None</td>
<td>155</td>
</tr>
<tr>
<td>27</td>
<td>46/M</td>
<td>M</td>
<td>CML, blast crisis</td>
<td>74,400</td>
<td>65% MB, 8% M, 13% B, 12% L</td>
<td>SBB-positive</td>
<td>Philadelphia chromosome present</td>
<td>None</td>
</tr>
<tr>
<td>28</td>
<td>60/M</td>
<td>M</td>
<td>AML, myeloblastic</td>
<td>29,000</td>
<td>96% MB</td>
<td>SBB-positive</td>
<td>Evolved from subacute leukemia</td>
<td>None</td>
</tr>
</tbody>
</table>

ND, not determined; At L, atypical lymphocyte; L, lymphocyte; P, polymorphonuclear leukocyte; M, monocyte; Abn. M, abnormal monocyte; LB, lymphoblast; ProL, prolymphocyte; MB, myeloblast; ProM, promyelocyte; MC, myelocyte; MMC, metamyelocyte; B, basophil; PAS, periodic acid Schiff; SBB, Sudan black B; MP, myeloperoxidase; NSE, nonspecific esterase; TdT, terminal deoxynucleotidyl transferase; TRAMPCOL, combination chemotherapy for CML, blast crisis.20
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leukocytes. In light of the dual cholesterol supply mechanism discussed above, this enhanced cholesterol synthesis might be due to a defect in the ability of the leukemic cells to take up LDL through the receptor system. In this case, the endogenously synthesized cholesterol would be replacing the cholesterol normally derived from LDL, so that the total flux of cholesterol would be normal. Alternatively, enhanced cholesterol synthesis in the leukemic cells might be due to a primary increase in the utilization of cholesterol for membrane synthesis or to an enhanced efflux of the sterol from cell membranes, in which case the rate of uptake of LDL might be normal or even elevated in the presence of an increased rate of cholesterol synthesis.

The current studies were therefore undertaken to measure the receptor-mediated uptake process for LDL in cells obtained from patients with different forms of human leukemia. As an index of LDL receptor activity, we measured the rate of high-affinity degradation of \(^{125}\text{I}-\text{LDL}\), a process that in normal cells is dependent upon the receptor-mediated binding, uptake, and lysosomal hydrolysis of the lipoprotein. For comparative purposes, we also studied LDL receptor activity in mixed mononuclear cells isolated from the blood of healthy subjects, patients with infectious mononucleosis, and patients with nonhematologic malignancies. The results show that the measured rate of receptor-mediated degradation of LDL was several times higher in peripheral mononuclear cells from patients with acute myelogenous leukemia (AML) compared with the other groups. Moreover, in the AML cells the total input of cholesterol (i.e., the sum of endogenously synthesized plus LDL-derived cholesterol) was ninefold higher than in the cells from the healthy subjects: 90\% of this input was derived from receptor-mediated uptake of LDL.

MATERIALS AND METHODS

Materials. \(^{125}\text{I}-\text{sodium iodide (carrier-free)}\) and \(^2\text{H}-\text{acetic acid, sodium salt (55 -59 mCi/mmol)}\) were purchased from Amersham/Searle. Lymphoprep, a mixture of 9.6\% sodium metrizoate and 5.6\% Ficoll (final density 1.077 g/ml), was obtained from Accurate Chemical and Scientific, 1-Hicksville, N.Y. Tissue culture supplies and chemicals for assays were obtained from sources as previously reported.

Subjects. The healthy subjects consisted of 26 normal individuals (17 males, 9 females); their mean age was 30 yr (range 12-65). Each normal subject had a plasma cholesterol level below 220 mg/dl, except for one 47-yr-old male whose value was 248 mg/dl. Each normal subject had a normal total and differential white blood cell count. In 14 of the healthy subjects, measurements of both \(^{125}\text{I}-\text{LDL degradation and cholesterol synthesis were made on the same blood sample. In the others, measurements of either one or the other process were performed. The clinical and hematologic data on the subjects with infectious mononucleosis, nonhematologic malignancies, and leukemia are summarized in Table I. Informed consent was obtained from each subject or from the parents.}

Lipoproteins. Human LDL (density 1.019-1.063 g/ml), high-density lipoprotein (HDL) (density 1.085-1.215 g/ml), and lipoprotein-deficient serum (density >1.215 g/ml) were obtained from the plasma of individual healthy subjects and prepared by differential ultracentrifugation as previously described. \(^{125}\text{I}-\text{LDL was prepared as previously described.}

Isolation of mononuclear cells. Blood mononuclear cells were isolated from heparinized venous peripheral blood by centrifugation through Lymphoprep as previously described. Cells at the interface were >95\% viable by the criterion of erythrosin B exclusion. The yield of mononuclear cells ranged from 88\% to 94\% of the number originally present in whole blood. The isolated mononuclear cells from the healthy subjects consisted of a mixture of lymphocytes (85\%-95\% of...
total cells) and monocytes (5%–15% of total cells) as determined by the functional criterion of polystyrene particle ingestion. The isolated mononuclear cells from the patients with acute leukemia consisted of a mixture of immature cells (lymphoblasts, myeloblasts, promyelocytes, or abnormal monocytes) and mature cells (lymphocytes and monocytes) in proportion to their occurrence in whole blood (Table I).

Assays. In most experiments receptor-mediated degradation of 125I-LDL by mononuclear cells was measured immediately after the cells were isolated from the bloodstream (method B, ref. 5). In one experiment the degradation was measured before and after the cells were incubated for 72 hr in lipoprotein-deficient serum (method A, ref. 5). In one experiment the total cellular uptake of 125I-LDL (receptor-bound plus internalized) at 37°C was determined as previously described.3 The rate of incorporation of 2-14C-acetate into cholesterol by mononuclear cells was measured by thin-layer chromatography as previously described.4 Incubations for the above assays were conducted at 37°C in a 5% CO2 incubator in a final volume of either 1 or 2 ml medium A (RPMI-1640 medium with penicillin [100 U/ml], streptomycin [100 μg/ml], and 10% or 30% human lipoprotein-deficient serum) as described in the cited articles. All incubations were carried out under conditions in which the amount of 125I-LDL degraded and the amount of 14C-acetate incorporated into 14C-cholesterol were proportional to both the duration of incubation and the number of cells. For all of the cell types studied, 10^6 cells contained 20–50 μg total cell protein.

The content of free and esterified cholesterol of mononuclear cells was determined as previously described.4 Protein was determined by the method of Lowry et al.13 with bovine serum albumin as a standard. Total and differential leukocyte counts were performed according to standard clinical hematology techniques using a Coulter counter and Wright-stained smears. The concentration of total cholesterol in plasma was measured by the cholesterol oxidase method (Boehringer Mannheim). The presence of the heterophile antibody of infectious mononucleosis was determined qualitatively using the Monospot Slide Test (Ortho Diagnostics). Histochemical staining of mononuclear cells—myeloperoxidase, sudan black B, and periodic acid Schiff—was performed by standard methods.14 T cell markers were assessed by observing the formation of spontaneous rosettes with sheep red blood cells15 and by staining with rabbit anti-human thymocyte antiserum as monitored by indirect immunofluorescence.15 Dr. Thomas Rogoff kindly measured enzymatic activity of lysozyme by a turbidometric method using the Lysozyme Reagent Set (Worthington Diagnostics). The activity of terminal deoxynucleotidyl transferase, which is a marker for acute lymphocytic leukemia cells, was determined as previously described.15

RESULTS

Table I presents the clinical features of the patients in the current study. Subjects 1–6 had infectious mononucleosis with positive heterophile agglutinins; subjects 7–13 had nonhematologic malignancies; subjects 14–18 had chronic lymphocytic leukemia (CLL) with 55%–99% mature lymphocytes on peripheral smear; subjects 19–21 were young individuals with acute lymphocytic leukemia (ALL) with 83%–92% lymphoblasts on peripheral smear; subjects 22–28 had AML of either myelomonocytic or myeloblastic type (two of these were patients with chronic myelogenous leukemia in blast crisis). In addition to the patients in Table I, a group of 26 healthy individuals was studied. In all subjects the total mononuclear cells were isolated from peripheral blood by sedimentation through Lymphoprep. This step, coupled with the subsequent washing, removed red blood cells, mature polymorphonuclear leukocytes, and most of the platelets, leaving a population of cells that was similar to the total mononuclear component of peripheral blood including mature cells (lymphocytes and monocytes), blasts, and other immature cells.16

To assess LDL receptor activity in the mononuclear cells, we measured the rate of high affinity 125I-LDL degradation. Previous studies in a variety of cell types have shown that this degradation rate is proportional to the number of
LDL receptors.135 Inasmuch as the degradation assay measures a catalytic property of the LDL receptor, this assay is at least 20 times more sensitive than the direct assay of 125I-LDL binding.37 To document the association between receptor-mediated 125I-LDL uptake and 125I-LDL degradation in acute leukemic cells, we measured both of these parameters in cells from a patient with AML (no. 22). Figure 1A shows the cellular content of 125I-LDL in freshly isolated mononuclear cells from this AML patient plotted as a function of the concentration of 125I-LDL that was present during a 4-hr incubation. The data show evidence for a saturable uptake process, with half-maximal uptake occurring at a 125I-LDL concentration of approximately 30 μg/ml. Similarly, when the amount of 125I-LDL degraded to trichloracetic acid-soluble radioactivity was measured, a saturable process was observed, with a half-maximal concentration of about 25 μg/ml 125I-LDL (Fig. 1B). These saturation curves for freshly isolated leukemic cells are similar to the saturation curves for receptor-mediated 125I-LDL uptake and degradation in human fibroblasts,17 lymphoblasts,18 and freshly isolated lymphocytes35 incubated for several days in the absence of LDL so as to induce a high level of LDL receptor activity.
Fig. 3. High-affinity degradation of $^{125}$I-LDL in freshly isolated mononuclear cells from healthy subjects and patients with infectious mononucleosis (inf. mono.), nonhematologic malignancies (cancer patients), CLL, ALL, and AML. Approximately $4 \times 10^9$ mononuclear cells were incubated in 1 ml medium A with 30% lipoprotein-deficient serum and 25 µg protein/ml of $^{125}$I-LDL (200-496 cpm/ng) in absence or presence of 500 µg protein/ml of unlabeled LDL. After incubation for 4-6 hr at 37°C, content of $^{125}$I-labeled acid-soluble (noniodide) material formed by cells and released into medium was determined. High-affinity (i.e., receptor-mediated) degradation of $^{125}$I-LDL was calculated as described in Results. Each data point, mean of triplicate incubations performed on cells from single subject. Number to right of each value, subject no. (Table 1).

To test the specificity of the receptor-mediated uptake and degradation process, we incubated the mononuclear cells from the same AML patient with 25 µg/ml $^{125}$I-LDL in the presence of increasing concentrations of unlabeled LDL or HDL. Whereas unlabeled LDL competitively inhibited the degradation of $^{125}$I-LDL by 50% at a concentration of approximately 100 µg protein/ml, unlabeled HDL did not inhibit significantly, even at concentrations as high as 500 µg protein/ml (Fig. 2).

Figure 3 compares the rates of high-affinity degradation of $^{125}$I-LDL in freshly isolated mononuclear cells obtained from the various subjects in the current study. Assays were performed under a standard set of conditions using $^{125}$I-LDL at 25 µg protein/ml. The high-affinity degradation represents that component of the total degradation due to the receptor-mediated uptake process. It was determined by subtracting the degradation of $^{125}$I-LDL in the pres-
ence of a 20-fold excess of unlabeled LDL (nonspecific degradation) from the degradation observed in the absence of unlabeled LDL (total degradation). Values for nonspecific degradation were always less than 20% of the values for total degradation.

Among 18 healthy subjects, the mean rate of high-affinity $^{125}$I-LDL degradation was 0.35 ng/hr/10^6 cells (range 0.22–0.56). This mean value is similar to the one previously reported in a smaller series of normal subjects. Mononuclear cells from patients with infectious mononucleosis and nonhematologic malignancies (cancer patients) tended to have slightly higher mean rates of $^{125}$I-LDL degradation (0.57 and 0.51 ng/hr/10^6 cells, respectively). Two of the five patients with CLL had degradation rates that were above the range seen in the normal cells (0.93 and 1.1 ng/hr/10^6 cells). The rate of $^{125}$I-LDL degradation was not elevated in cells from any of the three patients with ALL (Fig. 3).

Mononuclear cells from all of the patients with AML had rates of $^{125}$I-LDL degradation that were several times the range seen in the healthy subjects. Six
of the seven subjects showed degradation rates that were moderately to markedly elevated (0.9-3.3 ng/hr/10⁶ cells). One patient had a rate that was more than 100 times the mean in the normal cells (38 ng/hr/10⁶ cells). This patient (no. 24) was a 27-yr-old-male with AML who had a total white blood cell count of 17,800/mm³ with 74% abnormal monocytes and 7% myeloblasts on smear. This distribution of mononuclear cells did not differ significantly from that of one other AML patient (no. 22) whose ²⁵I-LDL degradation rate was ten-fold lower. The relatively higher rate of ²⁵I-LDL degradation in the AML group as compared to the other groups was the same when the data were expressed in terms of ng degraded/hr/mg cellular protein.

We also measured the rate of cholesterol synthesis from ¹⁴C-acetate in the mononuclear cells of the subjects in the current study. The data in Fig. 4 show that the rate of cholesterol synthesis in cells from all of the patients with infectious mononucleosis was three to six times that seen in the normal mononuclear cells. In contrast, cells from the patients with nonhematologic malignancies and with CLL had rates of cholesterol synthesis that were similar to those of the healthy subjects. Cells from all of the patients with ALL had cholesterol synthetic rates that were above those seen in normal mononuclear cells (two about 4 and one nearly 70 times values for normal cells). Similarly, cholesterol synthesis was accelerated in the mononuclear cells from all of the patients with AML, with five of the seven patients having values in the range 7-30 times those seen in the normal cells (Fig. 4).

Table 2. Degradation of ²⁵I-LDL and LDL-Mediated Suppression of Cholesterol Synthesis in Normal and Leukemic Cells

<table>
<thead>
<tr>
<th>Subject Providing Cells</th>
<th>High-Affinity Degradation of ²⁵I-LDL (ng/hr/mg protein) After 72-hr Incubation</th>
<th>Zero Time Without LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td>D.A. 3.8 174 30 514 87 38 26 24 ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R.G. 2.3 194 29 711 145 73 47 36 ND</td>
<td></td>
</tr>
<tr>
<td>ALL patient</td>
<td>No.19 2.9 29 3310 4370 ND ND 1110 840 568</td>
<td></td>
</tr>
<tr>
<td>AML patients</td>
<td>No.24 150 587 144 1324 ND 191 132 110 ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No.28 12 103 119 6670 ND 1140 ND 253 ND</td>
<td></td>
</tr>
</tbody>
</table>

Mononuclear cells were isolated from 30 ml venous blood from the indicated subject. Dishes of cells containing 2-4 x 10⁶ cells in a final volume of 2 ml medium A with 10% lipoprotein-deficient serum were prepared and studied at zero time or incubated at 37°C for 72 hr in the absence or presence of LDL as described for method A, ref. 5. For assays of ²⁵I-LDL degradation, each dish received 10 pg protein/ml of ²⁵I-LDL (165-246 cpm/ng protein) in the absence or presence of 250 pg protein/ml of unlabeled LDL. After incubation for 6-9 hr at 37°C, the content of ²⁵I-labeled acid-soluble (noniodide) material formed by the cells and released into the medium was determined. The high-affinity values were calculated as described in Results. For assays of cholesterol synthesis, cells were incubated for 4 hr with 2.5 mM ¹⁴C-acetate (29-60 cpm/pmol), and the synthesized ¹⁴C-cholesterol was measured as described in Materials and Methods. In some dishes, the indicated concentration of unlabeled LDL was added at zero time and cholesterol synthesis was measured after incubation for 72 hr. Each value for ²⁵I-LDL degradation and cholesterol synthesis represents the average of duplicate incubations. ND, not determined.
To study the regulation of the LDL receptor and cholesterol synthesis in the leukemic cells, we divided the freshly isolated cells into several aliquots. For one aliquot, we measured $^{125}$I-LDL degradation and cholesterol synthesis at zero time. The remaining aliquots were then incubated for 72 hr in the presence of varying concentrations of LDL and the measurements were repeated (Table 2). These experiments were conducted in cells from two normal subjects, one subject with ALL who had an extremely high rate of cholesterol synthesis at zero time, and two patients with AML whose cells showed moderate to markedly accelerated rates of $^{125}$I-LDL degradation at zero time. As previously shown,3-5 in the normal cells the rate of high-affinity degradation of $^{125}$I-LDL increased markedly when the cells were incubated for 72 hr in the absence of LDL. The induced 72-hr values for the two healthy subjects were similar to the values observed in 32 healthy subjects studied under identical conditions; the range of values for these 32 subjects was 92–312 ng/hr/mg (mean 149) (see Table III of ref. 5). In the ALL cells, induction of LDL receptor activity occurred, but the absolute rate of $^{125}$I-LDL degradation attained in the cells from this patient (29 ng/hr/mg) was about fivefold lower than the mean value observed in normal cells (149 ng/hr/mg). In cells from the two patients with AML, the rate of $^{125}$I-LDL degradation also increased significantly during the 72-hr incubation in the absence of LDL. This finding suggests that the high level of LDL receptors observed at zero time does not represent the maximal number of receptors that the cells are able to produce and that even in these AML cells the production of LDL receptors is under a degree of suppression by LDL in vivo (see below).

In both normal and AML cells the rate of cholesterol synthesis increased considerably when the cells were incubated for 72 hr in the absence of LDL (Table 2). In both normal and AML cells the inclusion of LDL in the incubation medium during the 72-hr incubation prevented the induction of cholesterol synthesis. The maximally effective concentration of LDL was in the range of saturation of the LDL receptor (i.e., less than 100 $\mu$g/ml). These data indicate that the LDL receptors present in the AML cells were active and that they were responsible for the partial suppression of cholesterol synthesis observed in these cells when they were freshly isolated.

The situation in the ALL cells was somewhat different. In these cells, the rate of cholesterol synthesis at zero time was relatively high, whereas the number of LDL receptors was relatively low (Table 2). Cholesterol synthesis rose by less than 25%, when the cells were incubated in the absence of LDL. The addition of LDL during the 72-hr incubation caused a suppression of cholesterol synthesis to levels below the initial value. However, the lowest rate of cholesterol synthesis attained (568 pmol/hr/mg protein) was still markedly above the rates in the normal and AML cells.

To study the regulation of the LDL receptor in AML cells in more detail, we incubated mononuclear cells from an additional AML subject for 67 hr in the absence or presence of LDL. The data in Table 3 show that the rate of high-affinity degradation of $^{125}$I-LDL rose by 15-fold when the cells were incubated in the absence of LDL. The inclusion of LDL in the incubation medium prevented this induction of LDL receptor activity. A similar prevention was
Table 3. Regulation of Receptor-Mediated Degradation of $^{125}$I-LDL in AML Cells by Inclusion of LDL or Sterols in the Incubation Medium

<table>
<thead>
<tr>
<th>Prior Treatment of Cells</th>
<th>Duration of Prior Incubation (hr)</th>
<th>$^{125}$I-LDL Degraded (ng/hr/mg protein)</th>
<th></th>
<th></th>
</tr>
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<tr>
<td>None</td>
<td>0</td>
<td>27</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>67</td>
<td>396</td>
<td>366</td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>67</td>
<td>51</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>25-Hydroxycholesterol + cholesterol</td>
<td>67</td>
<td>31</td>
<td>30</td>
<td></td>
</tr>
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</table>

Mononuclear cells were isolated from 30 ml venous blood obtained from patient 27. Four replicate stock flasks containing $30 \times 10^6$ cells in a final volume of 15 ml medium A (see ref. 5) with 10% lipoprotein-deficient serum were prepared. Each flask received one of the following additions: none, 100 µg protein/ml of unlabeled LDL, or 1 µg/ml of 25-hydroxycholesterol plus 10 µg/ml of cholesterol added in 35 µl of ethanol. After incubation at 37°C for either 0 or 67 hr, the cells from each flask were harvested by centrifugation (200 g, 10 min, 24°C), washed in 15 ml medium A containing 10% human lipoprotein-deficient serum, and recentrifuged. Each cell pellet was then resuspended in 10 ml medium A containing 10% human lipoprotein-deficient serum, and the resulting suspension of cells was divided equally into five 2-ml aliquots. Each 2-ml aliquot was transferred to a Petri dish to which was then added 10 µg protein/ml of $^{125}$I-LDL (85 cpm/ng) in the absence (three dishes) or presence (two dishes) of 250 µg protein/ml of unlabeled LDL. After incubation for 5 hr at 37°C, the content of $^{125}$I-labeled acid-soluble (noniodide) material formed by the cells and released into the medium was determined. The high-affinity values were calculated as described in Results.

*achieved when the cells were incubated with a mixture of 25-hydroxycholesterol plus cholesterol. This combination of sterols has been shown to prevent induction of LDL receptor activity in normal lymphocytes. These data support the conclusion derived from the data in Table 2, i.e., that the enhanced level of LDL receptor activity in the AML cells is not due to a loss of sensitivity to regulation by LDL-cholesterol.

Table 4 compares the cholesterol content in mononuclear cells from healthy

Table 4. Content of Cholesterol in Freshly Isolated Mononuclear Cells Obtained From Healthy Subjects and Patients With Acute Leukemia

<table>
<thead>
<tr>
<th>Subject Providing Cells</th>
<th>Cholesterol Content (µg Sterol/mg Protein)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free</td>
<td>Esterified</td>
<td></td>
</tr>
<tr>
<td>Healthy subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.G.</td>
<td>38</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>D.B.</td>
<td>28</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Nine subjects</td>
<td>33 (21–46)*</td>
<td>0.64 (0.36–1.03)</td>
<td></td>
</tr>
<tr>
<td>ALL patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 20</td>
<td>8.6</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>No. 21</td>
<td>9.2</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>AML patients</td>
<td></td>
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<tr>
<td>No. 22</td>
<td>16</td>
<td>0.13</td>
<td></td>
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<tr>
<td>No. 23</td>
<td>10</td>
<td>0.14</td>
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</table>

Mononuclear cells were isolated from 10 ml venous blood obtained from each of the indicated subjects. The cellular content of free and esterified cholesterol was determined as described in Methods and Materials. Each value represents the average of duplicate determinations.

*Mean and range of normal values previously reported from our laboratory (Table II, ref. 4).
subjects, two patients with ALL, and two patients with AML. As previously observed by others, the cellular content of cholesterol, as reflected by the cholesterol:protein ratio, was diminished in the cells from the four patients with acute leukemia as compared with the normal cells.

DISCUSSION

The major conclusion of the current studies is that mononuclear cells from patients with AML have a relatively high rate of receptor-mediated uptake and degradation of \( ^{125}\)I-LDL. The rates are much higher than those seen in mononuclear cells from healthy subjects and patients with infectious mononucleosis, various nonhematologic malignancies, CLL, and ALL. The high rate of degradation of LDL in the AML cells was associated with a relatively high rate of cholesterol synthesis in these cells, which was reported previously by several other laboratories.

In this study, as in all studies in which malignant cells are examined, a problem arose as to the appropriate normal cells with which to compare the results. In order to have a fixed reference point for the current studies, we measured \( ^{125}\)I-LDL degradation and cholesterol synthesis in mononuclear cells isolated from healthy subjects and patients with nonhematologic malignancies. These normal mononuclear cells represent a mixture of mature lymphocytes and monocytes. It seems unlikely that differences between the mononuclear cells from the leukemic patients and those from the healthy subjects were due to variations in the percentage of normal lymphocytes and monocytes. Previous studies have shown that when normal mononuclear cells are studied without prior incubation in the absence of lipoproteins, the rates of cholesterol synthesis are similarly low in both lymphocytes and monocytes. For technical reasons it has not yet been possible to compare directly the rates of \( ^{125}\)I-LDL degradation in monocytes and lymphocytes. However, in the current study the percentage of monocytes among total mononuclear cells of the healthy subjects varied from 5% to 15%, and no correlation was seen between the percentage of monocytes and the rate of \( ^{125}\)I-LDL degradation. Moreover, when isolated normal mononuclear cells were incubated in the absence of lipoproteins for 72 hr, LDL receptor activity was similar in nonadherent cells (mostly lymphocytes) and adherent cells (mostly monocytes). Thus the abnormalities in the leukemic blood samples are likely to be due to the presence of immature and malignant cells rather than to the presence of altered proportions of normal blood cells.

The current data cannot be interpreted as demonstrating that the higher rates of \( ^{125}\)I-LDL degradation and cholesterol synthesis are secondary to the leukemic process per se. It is possible, and perhaps even likely, that the behavior of the AML cells reflects the usual behavior of myeloid precursors in the bone marrow. Nevertheless, the measurement of LDL degradation and cholesterol synthesis in the normal and leukemic cell populations provides insights into the behavior of cells that display varying patterns of cholesterol homeostasis.

Inasmuch as the degradation of 1 ng \( ^{125}\)I-LDL protein yields about 1.6 ng cholesterol for cellular utilization, we were able to estimate the total amount of cholesterol derived from \( ^{125}\)I-LDL degradation per hr under the standard conditions in vitro in cells from the different patients. We also calculated the
mass of cholesterol synthesized from $^{14}$C-acetate per hr in the same cells. The data in Fig. 5 show that the total rate of input of cholesterol from these two sources$^{23}$ (i.e., the sum of LDL-derived cholesterol plus endogenously synthesized cholesterol) was approximately nine times higher in the AML cells than in the cells from the healthy subjects. This high rate was apparent even though we excluded from the calculations the cells from the one AML patient (no. 24) who had a $^{125}$I-LDL degradation rate ten times higher than that of the other six AML patients (Fig. 3).

The reason for the high rate of input of cholesterol into the AML cells remains to be elucidated. Previously workers have shown a relatively low content of free cholesterol and a low ratio of cholesterol:phospholipid in the blood mononuclear cells isolated from patients with a variety of acute leukemias.$^{19,20}$ The data in Table 4 also show a 50%, 75% lower cholesterol content of mononuclear cells from 2 patients with AML and 2 patients with ALL as compared with the cells from 11 healthy subjects. These data suggest that the enhanced cellular input of cholesterol in the AML cells occurs as a compensatory response to forces that lower the cholesterol content of the cell. One source of such drainage of cholesterol might be an enhanced rate of membrane synthesis associated with a more rapid rate of cell proliferation in the AML leukocytes as compared with normal mononuclear cells. Alternatively, the AML cells might actually have an enhanced rate of efflux of cholesterol from cell membranes, perhaps as a result of an accelerated rate of membrane turnover. In response to the loss of cholesterol, the AML cells develop an increase in LDL receptor activity. However, the number of LDL receptors is not high enough to supply all of the cholesterol needed by the cell, and hence cholesterol synthesis also increases.

Cells from patients with ALL also have a relatively low content of cholesterol. In contrast to the AML cells, however, the ALL cells respond to this cholesterol deficiency primarily by increasing the rate of cholesterol synthesis rather than developing an increased number of LDL receptors. The total rate of input of cholesterol in the ALL cells, although relatively high, is not as high as in the AML cells. This suggests that the rate of loss of cholesterol from the...
cell may be less in the ALL cells than in the AML cells. Whether this lower rate of loss accounts for the failure to induce LDL receptors is not known.

An important feature of cholesterol homeostasis in normal mononuclear cells is illustrated by the data in Fig. 5. For every 10⁶ normal cells, a total of 640 pg cholesterol was derived per hr from LDL degradation and cholesterol synthesis. Of this total, 620 pg/hr (97%) was derived from LDL degradation and only 3% from cellular cholesterol synthesis. These data are consistent with a large body of previous evidence in experimental animals indicating that extrahepatic cells synthesize only small amounts of cholesterol and derive the bulk of their cholesterol from circulating lipoproteins.²⁴⁻²⁶ As in the normal cells, in the AML cells more than 90% of the total cholesterol input was derived from LDL degradation. It should be noted that the measured uptake of LDL in these studies represents an underestimate of the true uptake rate for LDL in the body, since a nonsaturating level of LDL was used (25 μg/ml). It is likely that the true uptake rate for LDL cholesterol in the body is about twofold higher than the rate observed in vitro, and hence the percentage of cellular sterol derived from LDL may be somewhat higher than that shown in Fig. 5.

The total daily input of cholesterol from cholesterol synthesis and LDL degradation in normal mononuclear cells amounts to a relatively small fraction of the total cholesterol content of the cell. In normal mononuclear cells, the total cholesterol content is approximately 1150 ng/10⁶ cells. In the current experiments the total cholesterol input from LDL uptake and endogenous synthesis per 10⁶ cells was 640 pg/hr or 15 ng/24 hr. As discussed above, it is likely that the rate of LDL uptake in the bloodstream is about twofold higher than observed in vitro owing to the nonsaturating concentration of LDL employed. Thus in the body the normal mononuclear cell is taking up and synthesizing an amount of cholesterol equal to about 30 ng/10⁶ cells daily or 2.5% of the cellular content per day. In the AML cells, the total input is much higher, amounting to approximately 50% of the cellular cholesterol content per day. It should be noted that these figures represent only the unidirectional uptake derived from LDL degradation and endogenous cholesterol synthesis. In addition, the cholesterol of the mononuclear cell membrane is likely to exchange with the free cholesterol of plasma lipoproteins, as it does in macrophages²⁷ and other cell types.²⁸ Since this exchange process is usually equal in both directions, it does not account for any net accumulation of cholesterol.²⁸

An unexpected finding in the current studies was the fivefold higher rate of cholesterol synthesis in mononuclear cells from patients with infectious mononucleosis when compared with mononuclear cells from healthy subjects. The majority of the atypical lymphocytes that accumulate in this disease are thought to be derived from T lymphocytes.³¹ Whether these data indicate that normal T lymphocytes have higher rates of cholesterol synthesis than other normal mononuclear cells or that the rate of cholesterol synthesis rises in these cells or in other mononuclear cells during the course of infectious mononucleosis remains to be determined. The description in the current studies of various abnormal patterns of lipoprotein and cholesterol metabolism in cells from patients with leukemia and infectious mononucleosis should provide the basis for further studies designed to disclose the mechanisms underlying these regulatory phenomena.
LDL RECEPTORS IN LEUKEMIA CELLS

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Low-density lipoprotein (LDL) receptor activity in human acute myelogenous leukemia cells

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