Increased 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Activity and Cholesterol Biosynthesis in Freshly Isolated Hairy Cell Leukemia Cells

By Stanley Yachnin and Valsa Mannickarottu

Freshly isolated hairy cells from the peripheral blood of patients with hairy cell leukemia (HCL) synthesize 3-5-fold greater amounts of cholesterol, lanosterol, and squalene from [1-14C]-acetate than do normal human peripheral blood mononuclear cells under basal conditions of culture (i.e., in the presence of low-density lipoprotein). HCL cells also exhibit an eightfold increase in the activity of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase. These changes cannot be ascribed to increased rates of cellular proliferation in the HCL cells, nor are they a consequence of an increased rate of loss of newly synthesized cholesterol into the culture medium. The increased rate of cholesterol biosynthesis in HCL cells may result in an increase in their total cellular cholesterol content, as well as in an increase in their plasma membrane cholesterol-phospholipid molar ratio. These changes, in turn, are probably responsible for some of the clinical manifestations of this disease.

LEUKEMIC CELLS from patients with chronic lymphocytic leukemia have a low cholesterol content when compared with normal peripheral blood mononuclear cells (PBMC) and display a rate of cellular cholesterol biosynthesis from acetate [a measure of cellular 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity] that does not differ from that of PBMC. In contrast, leukemic cells from patients with hairy cell leukemia (HCL), another chronic B lymphocyte proliferative disorder, contain increased amounts of cholesterol compared to their normal counterparts, and display an even more striking increase in their cholesterol content when compared to lymphocytes from patients with chronic lymphocytic leukemia.

Despite the fact that HCL cells, as well as PBMC, are indolent populations with regard to DNA synthesis and cellular proliferation, we have shown that HCL cells synthesize 5-6-fold greater amounts of cholesterol from [1-14C]-acetate than do PBMC. These studies were conducted during the last 6 hr of a 24-hr incubation in medium containing 20% lipoprotein-depleted serum (LDS), which, by virtue of the absence of exogenous low-density lipoprotein (LDL) and cholesterol, results in enhancement of incorporation of acetate into cholesterol in both normal PBMC and HCL leukemia cells. We also showed that the presence of 100 μg LDL throughout the 24-hr incubation period suppressed cholesterol formation from acetate by 60% in both PBMC and HCL cells, but did not abolish the marked difference in their rates of cholesterol synthesis.

The purpose of the present study was to verify the increased rate of cholesterol biosynthesis in HCL cells relative to PBMC by utilizing freshly isolated cells in an attempt to avoid possible artifactual changes in enzyme activities secondary to prolonged periods of incubation. In addition, because the use of labeled acetate as a cholesterol precursor suffers from an inability to control the intracellular pool size of unlabeled two-carbon fragments, we studied the activity of HMG-CoA reductase in freshly isolated HCL cells and PBMC and showed that, in keeping with their increased rate of cholesterol synthesis from acetate, HCL cells also contain elevated levels of this enzyme, which is considered to be the key regulatory enzyme in the cholesterol synthetic pathway.

MATERIALS AND METHODS

3-Hydroxy-3-methylglutaryl coenzyme A, RS-mevalonic acid lactone, cholesterol, lanosterol, and squalene were all from Sigma (St. Louis, MO). Human heat-inactivated AB serum, LDS, and human LDL (specific gravity 1.019-1.063 g/ml) were prepared as previously described. Sodium [1-14C]-acetate, 57 mCi/m mole, and RS-[5-3H(N)]-mevalonolactone were from the New England Nuclear Corp. (Boston, MA). [4-14C]-Cholesterol, and di-3-hydroxy-3-methyl[3-14C]-glutaryl coenzyme A, 56 mCi/m mole, were purchased from Amersham Searle Corp. (Arlington Heights, IL).

Peripheral blood cells from 6 patients with HCL were studied (see Table 1 for patient data). The methods used for the isolation of PBMC and HCL cells from peripheral blood, as well as those used for determining their ability to incorporate [1-14C]-acetate into nonsaponifiable lipids (NSL), cholesterol, lanosterol, and squalene have been described previously. In the current studies, [1-14C]-acetate incorporation into NSL and components of the cholesterol biosynthetic pathway of 5 x 10^6 cells was measured from 18 to 24 hr of culture in medium containing 20% LDS, as well as in replicate cultures to which 100 μg LDL protein/ml had been added at the
time of culture initiation. Similar studies were also done utilizing cultures of freshly isolated cells to which the isotope was added at the time of culture initiation and the cells harvested for analysis 6 hr later. In certain experiments, cells were also cultured in medium containing 20% whole serum in the place of LDS. In addition, as described earlier, a replicate set of cultures in LDS medium was containing 20% whole serum in the place of LDS. In addition, as described earlier, a replicate set of cultures in LDS medium was included in many experiments in which the entire culture contents (including the medium) was subjected to saponification, lipid extraction, and thin-layer chromatography. The difference between the results obtained with these samples and those obtained using the washed cells was taken as a measure of the amount of newly synthesized cholesterol that passed from the cells to the external medium. A single preparation of LDS was utilized for all the studies presented in this report. All determinations of this kind were conducted in triplicate.

Ten million freshly isolated cells were washed 3 times (4°C) in phosphate-buffered saline (0.15 M NaCl, 0.01 M phosphate, pH 7.4) containing 10⁻⁶ M EDTA. The drained cell pellet was either subjected to HMG-CoA reductase assay immediately, or frozen at -80°C for later assay. Preliminary studies revealed that such storage, for a period of time as long as 5 mo, did not alter the assay results. All assays reported here were performed within 1 mo of the isolation and storage of cells. Determination of HMG-CoA reductase activity was performed in duplicate, utilizing the method of Kayden,* and the results are expressed as picomoles of substrate incorporated into mevalonolactone/minute/milligram protein. Protein determinations were done by a modification of the Lowry method. RS-[5-¹⁴C]-mevalonolactone was used as an internal standard to correct for losses of the ¹⁴C-mevalonolactone product during the extraction and thin-layer chromatography procedures used for its isolation. The final concentration of HMG-CoA (specific activity 10 µCi/µmole) in the assay mixture was 30 µM (±6.6 cpm/µmole). The standard deviation of the mean of duplicate assays of values >5 pmole/min/mg protein was ±5%–10% of the mean. Lower mean values had a somewhat higher SD.

HMG-CoA reductase activity was also measured in PBMNC that had been depleted (<2%) of monocytes by passage over a nylon fiber column and in sheep erythrocyte rosette-positive (E+) and rosette-negative (E-) cell populations prepared from the monocyte-depleted cell preparation. For experiments in which changes in HMG-CoA reductase activity during the period of culture were followed, 10⁶ cells were incubated in the media, already described, at a concentration of 2 x 10⁶/ml in upright 25 sq cm tissue culture flasks (Falcon, cat. no 3013, Oxnard, CA). At the conclusion of the incubation period, the cells were washed 4 times in cold phosphate-buffered saline, drained, and frozen at -80°C for later assay.

**RESULTS**

Incubation of normal PBMNC in LDS medium for 24 hr resulted in a 10.1 ± 5.0-fold (mean ± SD, n = 15) increase in HMG-CoA reductase activity; this increase could be entirely prevented by the presence of 100 µg LDL protein/ml or the replacement of LDS by 20% whole serum in the incubation medium (results not shown). The presence of LDL in LDS medium for 24 hr also kept the PBMNC in a basal state, as measured by [¹⁴C]-acetate incorporation into cholesterol and its two major precursors, lanosterol and squalene (Table 2). When, instead of adding LDL, LDS was replaced with 20% whole serum, similar results were obtained (data not shown).

Even in the basal state (0–6 hr incubation in LDS + LDL or in whole serum medium), HCL cells displayed a 3–5-fold increase in their ability to incorporate [¹⁴C]-acetate into cholesterol, lanosterol, and squalene when compared to PBMNC (Table 2). Both PBMNC and HCL cells increased their rate of cholesterol, lanosterol, and squalene synthesis over basal levels if incubated in the absence of lipoproteins and cholesterol during the first 6 hr of culture. PBMNC

---

Table 1. Clinical Data on HCL Patients and HMG-CoA Reductase Activity in HCL Cells and PBMNC

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Age</th>
<th>Sex</th>
<th>White Blood Cell Count* (cells/mm³)</th>
<th>Percent Hairy Cells*</th>
<th>HMG-CoA Reductase Activity (pmole/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>M</td>
<td>31,200</td>
<td>80-85</td>
<td>13.4 ± 3.4†‡ (5.2)§</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>M</td>
<td>12,800</td>
<td>62</td>
<td>9.8 ± 0.6 (2.3)</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>M</td>
<td>19,000</td>
<td>72</td>
<td>18.4 ± 0.4 (6.4)</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>M</td>
<td>31,800</td>
<td>89</td>
<td>10.7 ± 1.1 (4.8)</td>
</tr>
<tr>
<td>5</td>
<td>58</td>
<td>M</td>
<td>9,700</td>
<td>16</td>
<td>4.2 ± 0.1 (1.8)</td>
</tr>
<tr>
<td>6</td>
<td>70</td>
<td>M</td>
<td>92,300</td>
<td>94</td>
<td>11.9 ± 0.3 (4.5)</td>
</tr>
</tbody>
</table>

*The values for patient 1 represent mean ± SD of 4 determinations done on samples of his peripheral blood cells over a period of 5 mo. The values for other HCL cells represent the mean ± SD of a single duplicate assay.

†Range of individual values; n = number of determinations.

‡The values in parentheses represent HMG-CoA reductase activity expressed as pmole/min/10⁶ cells.

§The values in parentheses represent HMG-CoA reductase activity expressed as pmole/min/10⁶ cells.

The following were the percentages of lymphocytes (which would also be included in our HCL cell isolates) in the peripheral blood of the hairy cell patients studied: patient 1, 7%–13%; patient 2, 21%; patient 3, 27%; patient 4, 11%; patient 5, 66%; patient 6, 4% lymphocytes. The peripheral blood of all patients contained less than 5% monocytes. All patients had undergone splenectomy at least 1 yr prior to study.
lost approximately 30% of newly synthesized cholesterol to the incubation medium under these conditions, and HCL cells lost approximately 20% (data not shown), but the differences were not significant. As with normal PBMNC, the presence of LDL or whole serum during the 24-hr incubation of HCL cells prevented the increase in sterol pathway product formation, which otherwise ensued in LDS medium. Freshly isolated HCL cells contained from 3–13 times the HMG-CoA reductase activity as did normal PBMNC, with a mean increase of eightfold (Table 1).

HMG-CoA reductase activity was measured in monocyte-depleted normal peripheral blood lymphocytes, as well as in the E+ and E− subpopulations (Table 3). The B cell-enriched E− cells contained the highest HMG-CoA reductase activity. All but one (patient 5) of our HCL cell isolates possessed significantly higher HMG-CoA reductase activity than the E− cell population.

### DISCUSSION

In an earlier study we showed that following an 18-hr incubation in lipoprotein- and cholesterol-depleted medium, HCL cells incorporated 5–6 times more 14C-acetate into cholesterol than do normal PBMNC over the ensuing 6 hr. Similar results were obtained in the present study. In addition, the present studies confirm that our earlier findings were not simply a reflection of a greater tendency on the part of HCL cells to increase their rate of cholesterol synthesis when deprived of exogenous cholesterol, as they reveal that freshly isolated HCL cells, studied under basal conditions (in the presence of lipoproteins), still incorporate acetate into cholesterol and its precursors at a rate that is 3–5 times greater than normal PBMNC. In addition, direct measurement of HMG-CoA reductase specific activity in freshly isolated HCL cells provides further proof of the enhanced cholesterol biosynthetic capacity of HCL cells, as this key enzyme of cholesterol biosynthesis is eight times more active in HCL cells than in normal PBMNC.

The lowest HMG-CoA reductase value found among our HCL cell isolates was in that patient (no. 5) whose peripheral blood contained the lowest percentage of hairy cells, and whose cell isolate contained a preponderance of normal lymphocytes. As is usually the case, all of the HCL patients were monocytopenic, and their increased cellular HMG-CoA reductase activity was therefore not a result of significant monocyte contamination. Finally, our experiments with monocyte-depleted lymphocytes, separated into E+ and E− populations, allow comparison of HCL cells,
an abnormal B lymphocyte population, with various subpopulations of normal peripheral blood lymphocytes. All but one of our HCL cell samples possessed significantly higher HMG-CoA reductase activity than normal B cell-enriched E- lymphocytes.

Although increased rates of cholesterol biosynthesis are found in other forms of leukemia, the majority of these involve cells with a high rate of mitotic activity and DNA synthesis and a low cholesterol content or low cholesterol:phospholipid molar ratio. It has been suggested that the increased rate of cholesterol synthesis in such cells is a consequence of the need to endow daughter cells with cholesterol-containing membrane constituents or to compensate for a more rapid efflux of cholesterol from leukemic cells to the exterior. Our earlier and present studies show that the increased capacity of HCL cells for cholesterol biosynthesis cannot be a consequence of either of these mechanisms. As others have shown that HCL cells, by comparison with normal human peripheral blood lymphocytes, have a high cholesterol content and a high cholesterol:phospholipid molar ratio in their plasma membranes, we believe that the capacity of HCL cells for enhanced cholesterol biosynthesis may be directly involved in their pathophysiologic manifestations. Increased synthesis of cholesterol might lead to the production of hairy cells with redundant plasma membrane, whose increased cholesterol:phospholipid molar ratio could result in decreased membrane fluidity and deformability. Such rigid, nonplastic HCL cells might have difficulty in traversing the splenic sinusoids, resulting in splenic engorgement. Pharmacologic suppression of the increased rate of cholesterol biosynthesis in HCL cells by agents capable of inhibiting or suppressing HCL cell HMG-CoA reductase activity might result in a reversion of the abnormal surface properties of HCL cells that lead to their splenic sequestration.

ACKNOWLEDGMENT

We are grateful to Dr. Angelo M. Scanu, University of Chicago, for providing the lipoprotein-depleted serum and low-density lipoprotein, and to Dr. Harvey M. Golomb and coworkers, University of Chicago, for help in obtaining cells from patients with hairy cell leukemia.

REFERENCES

Increased 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and cholesterol biosynthesis in freshly isolated hairy cell leukemia cells

S Yachnin and V Mannickarottu