Stimulation of Sterol Synthesis in Peripheral Leukocytes of Leukemic Mice

Harry W. Chen and Hans-Joerg Heiniger

The Jackson Laboratory, Bar Harbor, Maine 04609

SUMMARY

Bone marrow cells and peripheral blood leukocytes from leukemic AKR/J mice synthesize sterols from acetate at a vastly greater rate than the respective cells from normal AKR/J mice, i.e., approximately 20- and 100-fold. The controlling enzyme of sterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase, is readily detectable in gradient-purified leukocytes from leukemic mice, whereas normal leukocytes, fractions of red blood cells, and platelets express very low activity. The increased rate of sterol synthesis in leukocytes and bone marrow cells of leukemic animals does not result in an elevated plasma cholesterol level.

INTRODUCTION

In a previous study (1), we reported that sterol synthesis was 10-fold greater in the thymus, spleen, and lymph nodes of leukemic mice than in those of normal AKR/J and HRS/J mice. Although the elevated sterol synthesis was accounted for by the increase of the regulatory enzyme HMG-CoA reductase (EC 1.1.1.34), the precise mechanism for the high rate of sterol synthesis in leukemic lymphatic tissues is not as yet known.

In this paper we show (a) that the blood and bone marrow cells from leukemic AKR/J mice also possess a very high sterol synthetic rate, (b) that this increase of sterol synthesis is mainly attributable to the leukocyte fraction in which HMG-CoA reductase activity is readily detectable, and (c) that no significant differences exist between circulating cholesterol levels in normal and leukemic mice.

MATERIALS AND METHODS

Mice. AKR/J mice were supplied by the Production Department of The Jackson Laboratory. They were given Old Guilford Chow 96W and water ad libitum. Leukemic mice ranged in age from 7 to 9 months. Leukemia was first diagnosed by clinical inspection, i.e., palpation for lymphadenopathy and splenomegaly, and later was verified grossly and by histological examination of tissues. Although we had not previously observed an age effect on the rate of sterol synthesis (1), control AKR/J mice 4 months old or less were used in order to eliminate any possibility of using preleukemic animals.

Analysis of Lipid Synthesis and CO₂ Production in Whole Blood. The mice were decapitated following asphyxiation in CO₂. One-half ml of whole blood was collected in sterile test tubes that contained 0.03 ml of sodium heparin, 1000 USP/ml (Sigma Chemical Co., St. Louis, Mo.). The blood was mixed and then added to a sterile Erlenmeyer flask containing 4.5 ml of RPMI 1640 medium (Grand Island Biological Co., Grand Island, N. Y.) and 0.1 ml of water containing 50 μCi (0.85 μmole) of sodium acetate-1⁻¹⁴C (New England Nuclear, Boston, Mass.). The flasks were sealed with septum stoppers fitted with plastic cups (Kontes Glass Co., Vineland, N. J.) and incubated in a shaking water bath for 2 hr at 37°C. The procedures for analysis of radioactive CO₂, fatty acids, and sterol fractions of the samples were similar to those described previously (1, 7, 9).

Purification of Blood Cells. Purification of leukocytes from blood was performed according to a modification of the method of Perper et al. (13). Approximately 7 ml of heparinized blood pooled from 8 animals were diluted with an equal volume of RPMI 1640 medium containing 24 mM N'-2-hydroxyethylpiperazine-N''-ethanesulfonic acid buffer (Grand Island Biological) and 0.5 mg EDTA per ml. This mixture was layered over a 10-ml Ficoll-Hypaque gradient and centrifuged at 500 × g for 25 min at 12°C. Ficoll was obtained from Sigma and Hypaque (sodium diatrizoate) was obtained from Winthrop Laboratory, New York, N. Y. Whereas the red blood cells sedimented to the bottom, the leukocytes appeared in an interphase layer; the platelets occurred between the supernatant plasma and interphase layers. Leukocytes were purified by withdrawal of about 8 ml from the interphase layer. They were washed and centrifuged once each in phosphate-buffered saline and in RPMI medium at 250 × g. The cells were then resuspended in the medium for acetate incorporation. Likewise, the red blood cells at the bottom of the tube were washed with phosphate-buffered saline, centrifuged, and resuspended in RPMI 1640 medium for analysis. For platelet studies, 20 ml of leukemic blood and 50 ml of normal heparinized blood were mixed with equal volumes of 0.9% NaCl solution and passed through columns of glass beads (diameter, 3.0 mm) to remove the “sticky” population of platelets. Effluents...
were then layered on a Ficoll-Hypaque gradient and fractionated as described above. The white cell mass containing leukocytes and platelets was removed and mixed with 2 volumes of 12\% sucrose gradient and centrifuged at 225 × g for 15 min at 12°. Thereafter, leukocytes sedimented to the bottom of the tubes, whereas the platelets remained in suspension; they were then collected and washed by centrifugation and used for acetate incorporation as described for the other cell types.

**Bone Marrow.** For analysis of sterol synthesis in bone marrow cells, 4 normal and 4 leukemic mice were killed by cervical dislocation. The femora and humeri were rapidly dissected out and collected separately in cold 0.9\% NaCl solution. Then the marrows were flushed from the respective bones with a total of 4 ml of RPMI 1640 medium and the use of a 26-gauge needle. The marrow was uniformly suspended in the medium and then was centrifuged at 160 × g for 10 min at 10°. The supernatant was discarded and the cells were carefully resuspended in 1 ml of RPMI 1640 medium. From this cell suspension, 0.2 ml was pipetted into 4 Erlenmeyer flasks containing 4 ml of RPMI 1640 medium and 50 μCi of acetate-14C. Then the cultures were processed according to standard procedures described above. One-tenth ml of the cell suspension was used for a Lowry (10) protein determination and 0.05 ml was used for a cell count in a standard Neubauer chamber.

**Plasma Cholesterol and Hematocrit Values.** For plasma cholesterol determination, the mice were bled from the orbital sinus with heparinized capillary tubes. These tubes were centrifuged in an International microcapillary Model MB centrifuge (International Equipment Co., Needham Heights, Mass.) for 10 min. Following hematocrit readings, the tubes were broken at the cell-plasma interphase and plasma was withdrawn with a micropipet. Plasma cholesterol was determined in 25 μl of samples by the micro-method of Poole (14) with a single stable commercial Libermann-Burchard reagent (15% by weight of sulfuric acid in an equal mixture of acetic acid:acetic anhydride; Hycel Inc., Houston, Texas).

**RESULTS**

Incorporation of acetate into sterol and fatty acids of bone marrow cells from leukemic AKR/J mice was stimulated up to 20 times, whereas acetate utilization for CO2 production was only moderately increased (Table 1). An even greater increase in sterol synthesis occurred in leukemic blood. Examination of 3 blood elements consisting of leukocytes, platelets, and erythrocytes revealed that the major increase of sterol synthesis was contributed by leukocytes (Table 2), although cellular fractionation was not complete. Whereas the leukocyte fraction contained mainly lymphocytes, granulocytes, and unidentified mononuclear cells, about one-quarter of the cells were erythrocytes. A few white blood cells were detected in both the platelet and red cell fractions from leukemic mice. This white cell contamination could have contributed to the apparent small increase of sterol synthesis in platelets and red cells of leukemic mice.

The kinetics of acetate-14C incorporation into sterol fractions of blood is shown in Chart 1. Normal blood...
**Table 2**  
Conversion of acetate to sterols, fatty acids, and CO2 by circulating elements of the blood

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Conditions</th>
<th>N</th>
<th>Sterols (dpm x 10^-5)</th>
<th>Fatty acids (dpm x 10^-3)</th>
<th>CO2 (dpm x 10^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td>Normal</td>
<td>5</td>
<td>0.1 ± 0.03*</td>
<td>0.9 ± 0.3</td>
<td>4 ± 2</td>
</tr>
<tr>
<td></td>
<td>Leukemic</td>
<td>5</td>
<td>1 ± 0.1</td>
<td>0.9 ± 0.3</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Platelets</td>
<td>Normal</td>
<td>3</td>
<td>1 ± 0.1</td>
<td>81 ± 7</td>
<td>32 ± 12</td>
</tr>
<tr>
<td></td>
<td>Leukemic</td>
<td>2</td>
<td>11</td>
<td>151</td>
<td>18</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>Normal</td>
<td>6</td>
<td>9 ± 3</td>
<td>83 ± 18</td>
<td>35 ± 13</td>
</tr>
<tr>
<td></td>
<td>Leukemic</td>
<td>10</td>
<td>716 ± 64</td>
<td>520 ± 69</td>
<td>178 ± 13</td>
</tr>
</tbody>
</table>

* Mean ± S.E./mg of protein, 2 hr of incubation; see "Materials and Methods" for experimental condition.

Table 3  
HMG-CoA reductase activity in leukocytes of normal and leukemic AKR/J mice

<table>
<thead>
<tr>
<th>Source</th>
<th>N</th>
<th>Specific activity of HMG-CoA reductase (nmoles MVA/mg protein/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4</td>
<td>0.05 ± 0.02*</td>
</tr>
<tr>
<td>Leukemic</td>
<td>3</td>
<td>0.37 ± 0.04</td>
</tr>
<tr>
<td>Leukemic: normal</td>
<td></td>
<td>7:1</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

There was no significant difference between the plasma cholesterol levels in the leukemic and normal mice (144 ± 13 mg/100 ml for the former and 163 ± 6 for the latter), but the hematocrit levels were significantly lower in leukemic than in normal mice (38.7 ± 1.5 and 51.5 ± 0.2%, respectively).

**DISCUSSION**

Fresh mouse blood readily incorporates radioactive acetate into sterols and fatty acids and utilizes it to produce CO2 through the Krebs cycle. The rate of sterol production in blood from normal mice is slow. In contrast, the blood obtained from leukemic animals exhibits a very high rate of sterol synthesis, a rate nearly 100-fold greater than that of normal blood. Although the rates of fatty acid synthesis and CO2 evolution in the leukemic blood are moderately stimulated as well, the extent of increase is far less than that of sterol synthesis. Therefore, the selective elevation of sterol synthesis in or during leukemogenesis relative to fatty acid and CO2 production cannot be accounted for simply by differences in acetate transport across cell membranes.

Since leukocytes, platelets, and erythrocytes from humans (11) and rabbit (17, 18) can synthesize lipid from...
acetate, it was necessary for us to identify the specific cell type(s) responsible for the elevated sterol synthesis in leukemic blood. Thus, we fractionated mouse blood by gradient centrifugation and found that (a) the increase of sterol synthesis in purified leukemic leukocytes was much greater than that of the normal leukocytes, and (b) the platelets and erythrocytes of normal mice possessed very low activity, and the small amount of activity observed in leukemic platelet and erythrocyte fractions probably derived from contamination of leukocytes in these fractions.

It is now well established that in mammals HMG-CoA reductase is the regulatory enzyme controlling the biosynthesis of cholesterol (9, 15). Analogous to our previous finding in leukemic mouse spleens (1), leukemogenesis resulted in an elevation of this enzyme in circulating leukocytes. Since leukemic cells in mice (12, 16) and in humans (2, 12, 16) exhibit neither increased proliferation nor shortened cell cycles, compared with the normal cells, the difference in sterol synthesis cannot be explained merely by differences in the rate of cell division.

The elevation of cholesterol synthesis has been demonstrated in various cancerous and precancerous tissues, e.g., spontaneous hepatoma in mice (8), Morris minimal deviation hepatomas in rats (3, 14), L2C transplantable leukemic in guinea pigs (15), and precancerous livers obtained from rats treated with hepatocarcinogens, either N-2-fluorenylacetamide (4, 5), aflatoxin (5), or ethionine (6). In the cases mentioned above, the increase of cholesterol synthesis in malignant or premalignant tissues was explained in terms of the loss of normal feedback control of cholesterol synthesis. Whether the loss of this regulatory mechanism also occurred in spontaneous leukemia in mice has yet to be determined. In any event, the mechanism leading to abnormal high production of cholesterol in cancer cells and its relationship to the process of malignant transformation is unknown.

ACKNOWLEDGMENTS

We thank Oral Applegate for competent technical assistance and Dr. Andrew Kandutsch and Dr. Hans Meier for valuable suggestions and critical review of the manuscript.

REFERENCES

Stimulation of Sterol Synthesis in Peripheral Leukocytes of Leukemic Mice

Harry W. Chen and Hans-Joerg Heiniger

*Cancer Res* 1974;34:1304-1307.

Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/34/6/1304](http://cancerres.aacrjournals.org/content/34/6/1304)