In vivo regulation of human leukocyte 3-hydroxy-3-methylglutaryl coenzyme A reductase: increased enzyme protein concentration and catalytic efficiency in human leukemia and lymphoma[1]

H. James Harwood, Jr.,[2,*,] Idania M. Alvarez,[*,**] Ward D. Noyes,[†] and Peter W. Stacpoole[*,**]**

Departments of Medicine (Division of Endocrinology and Metabolism* and Hematology and Oncology†) and Pharmacology,** University of Florida, College of Medicine Gainesville, FL 32610

Abstract The activity of microsomal HMG-CoA reductase in freshly isolated leukocytes from patients with a variety of hematologic malignancies was significantly increased (up to 20-fold) when compared to enzyme activity in leukocytes from normal subjects (average 10.3 ± 0.8 pmol/min per mg). Increased enzyme activity was not due to nonspecific leukocyte stimulation or to the presence of a malignancy, since normal enzyme activity was observed in subjects with either viral illnesses or solid tumors. Increased HMG-CoA reductase activity accompanying hematologic malignancy could also not be attributed to alterations in enzyme-substrate kinetic parameters (K_m), or to alterations in the phosphorylation state or thiol-disulfide status of the enzyme, nor was it correlated with differences in serum lipid or lipoprotein concentrations. The increase (3.6-fold) in HMG-CoA reductase activity in leukocytes from patients with preleukemia was due entirely to a rise in enzyme catalytic efficiency (specific activity), whereas the increase (4.3-fold) observed in leukocytes from patients with overt leukemia or non-Hodgkin's lymphoma was due to a concomitant increase in both enzyme catalytic efficiency (2.5-fold) and enzyme protein concentration (1.6-fold). Similar increases in HMG-CoA reductase activity and catalytic efficiency were also noted for both transformed, nonmalignant, and malignant cultured leukocytes, suggesting that increased enzyme catalytic efficiency is not a nonspecific consequence of physiological changes occurring in response to the malignancy but may be an integral aspect of the malignant phenotype. HMG-CoA reductase protein concentrations, however, were not elevated in either transformed, nonmalignant, or malignant cultured leukocytes, suggesting that increases in enzyme protein levels may be secondary to other physiological changes that occur during the development of overt leukemia. Taken together, these observations suggest that an increase in the activity of HMG-CoA reductase, the rate-controlling enzyme in cholesterol synthesis, is a common occurrence in human hematologic malignancies and that a biphasic elevation of enzyme activity may exist in malignant leukocytes, such that changes in catalytic activity may occur early in tumorigenesis and may be followed by secondary changes in enzyme levels. —**Harwood, H. J., Jr., I. M. Alvarez, W. D. Noyes, and P. W. Stacpoole.** In vivo regulation of human leukocyte 3-hydroxy-3-methylglutaryl coenzyme A reductase: increased enzyme protein concentration and catalytic efficiency in human leukemia and lymphoma. *J. Lipid Res.* 1991. **32:** 1237–1252.

Supplementary key words leukocytes • preleukemia • cholesterol • cultured leukocytes • cell growth • malignancy

For many years, a possible central role has been entertained for cholesterol in the pathobiology of cancer (1–5). Abnormalities in the synthesis (4–6), uptake (7–11), and membrane content (1, 4, 12) of cholesterol have been causally associated with the proliferation, if not the induction, of tumors.

Siperstein and Fagan (13) provided the first strong evidence for disordered regulation of cholesterol metabolism in cancer cells, and this finding has been substantiated repeatedly for both solid tumors (2–5) and hematologic malignancies (3, 4). In general, these tumors fail to exhibit normal feedback suppression of sterol synthesis in response to dietary or lipoprotein cholesterol (2, 4), possibly as a result of altered lipoprotein uptake by malignant cells (14–16), and thus express increased basal rates

Abbreviations: ALL, acute lymphocytic leukemia; ANLL, acute nonlymphocytic leukemia; FAB-M3, acute promyelocytic leukemia; FAB-M1 or M2, acute myelogenous leukemia; FAB-M4, acute monomyelocytic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; HMG, 3-hydroxy-3-methylglutaryl; TBS, Tris-buffered saline; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein.

[1]A preliminary report of portions of these studies was presented at the American Federation for Clinical Research Annual Meeting in April 1986 (91).

[2]To whom correspondence should be addressed at: Atherosclerosis Section, Department of Metabolic Diseases, Pfizer Central Research, Pfizer Inc., Eastern Point Road, Groton, Ct 06340

Downloaded from www.jlr.org by guest, on June 18, 2012

of cholesterol formation (2–5). Disordered regulation of cholesterol formation appears to be an early event in the development of malignancy, occurring within 2 weeks of exposure of rats to carcinogens (17) and several months before the appearance of hepatomas following exposure of trout to aflatoxin (5). Altered regulation is not simply a consequence of increased cellular proliferation, however, since the rapidly dividing cells of fetal and regenerating liver exhibit normal feedback responses to dietary cholesterol (5, 13).

The high rate of cholesterol production by solid tumors has been linked to an increase in the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase [E.C.1.1.1.34], the endoplasmic reticulum enzyme that catalyzes the rate-determining step in cholesterol synthesis (18). Although it is not known why HMG-CoA reductase activity is increased in cancer cells, increased enzyme activity persists even in cultured tumor cells that have regained their ability to suppress cholesterol synthesis through feedback regulation (3, 19), suggesting that enzyme activity may be increased through a mechanism independent of alteration in feedback suppression. The physico-chemical and kinetic properties of the enzyme isolated from tumors, however, appear very similar to those derived from normal tissues (20, 21). In two independent reports, it has been suggested that changes in the basal phosphorylation state of the enzyme are partly responsible for the increased HMG-CoA reductase activity in malignant tissues (22, 23), and in one report a dramatic increase in the cellular amount of HMG-CoA reductase protein in a solid rat hepatoma was comensurate with the hepatoma's increased cholesterol synthesis (24). To date, however, the exact mechanism for the increase in HMG-CoA reductase activity in malignant tissues has not been elucidated.

Preliminary evidence has suggested that HMG-CoA reductase activity may also be increased in human hematologic malignancies (6, 7, 25). Several laboratories have observed greatly increased rates of cholesterolgenesis from [^{14}C]acetate in freshly isolated leukocytes from patients with a variety of leukemias (6, 7, 25). In these studies, however, HMG-CoA reductase activity was not measured directly, but was implied through rates of sterol synthesis. Since normal granulocytes do not synthesis sterols (26, 27), and the majority of newly formed mevalonate in lymphocytes and monocytes is converted to nonsterol products (26, 27), changes in the rate of cholesterol synthesis from acetate in human leukocytes may not accurately reflect changes in leukocyte HMG-CoA reductase activity or in the production of nonsteroidal products of mevalonate metabolism.

To evaluate the effects of malignancy on the intrinsic properties of human leukocyte HMG-CoA reductase, we used recently developed techniques for directly measuring both HMG-CoA reductase activity (28) and HMG-CoA reductase protein concentration (29) and for calculating HMG-CoA reductase catalytic efficiency (specific activity) in microsomal fractions from either freshly isolated or cultured human leukocytes. Using these methods, we previously demonstrated that the regulatory properties of HMG-CoA reductase in normal human leukocytes qualitatively resemble those of normal animal tissues (30, 31).

In this report we examined HMG-CoA reductase activity in both trasnformed and malignant cultured human leukocytes and in leukocytes isolated from patients with either preleukemia, overt leukemia, or lymphoma. Our results show that the catalytic efficiency of human leukocyte HMG-CoA reductase is increased dramatically in the cells of most patients with either preleukemia, leukemia, or non-Hodgkin's lymphoma, and in all transformed malignant and nonmalignant cell lines examined, through mechanisms independent of alterations in enzyme-substrate kinetic parameters (K_m) or of changes in the phosphorylation state or thiol-disulfide status of the enzyme. Mechanisms responsible for the elevated catalytic efficiency of HMG-CoA reductase in patients with preleukemia appeared to be qualitatively different from those in subjects with frank leukemia or lymphoma.

MATERIALS AND METHODS

Subjects

This investigation was approved by the Institutional Review Board of Shands Hospital, Gainesville, Florida. Participants in this study involved 34 healthy normocholesterolemic individuals, 5 patients with preleukemic syndrome,[3] 6 with acute lymphocytic leukemia (ALL), 7 with acute nonlymphocytic leukemia (ANLL; acute promyelocytic leukemia (FAB-M3), n = 1; acute myelogenous leukemia (FAB-M1 or M2), n = 2; acute monomyelocytic leukemia (FAB-M4), n = 4), 9 with chronic lymphocytic leukemia (CLL), 1 with hairy cell leukemia, 8 with chronic myelogenous leukemia (CML; chronic myelogenous leukemia, n = 6; chronic monomyelocytic leukemia,[3] n = 2), 5 with non-Hodgkin's lymphoma, 4 with breast cancer, 1 with colon cancer, and 1 with choriocarcinoma. Individual patient information with respect to age, sex, disease and status, peripheral leukocyte count and differential, and current medication is presented in **Table 1**.

[3]The term "preleukemia" is used in this context in the manner set forth by Bagby (49) to encompass patients in the early stages of hemopoietic dysplasia. In this context, and to the extent to which CMMoL resembles overt leukemia, we have categorized our patients with CMMoL together with those possessing CML.

Downloaded from www.jlr.org by guest, on June 18, 2012

TABLE 1. Details of cancer patients studied

Patient Number	Sex	Age	Disease and Status[a]	Peripheral Blood Leukocyte Count	Percent Immature	Percent Blasts	Medications
		yr					
1	M	74	CMMoL, stable	3,600	2	0	none
2	F	73	CMMoL, accelerating	86,500	39	0	zentar
3	F	63	CML, accelerating	10,800	5	0	busulfan, prednisone
4	M	58	CML, blast crisis	25,000	13	9	prednisone, 6MP, vincristine
5	F	42	CML, stable	18,900	6	0	busulfan
6	M	78	CML, blast crisis	60,000	—[b]	—	—
7	M	50	CML, blast crisis	72,000	—	—	none
8	M	—	CML, accelerating	67,000	45	0	none
9	M	68	CLL, accelerating	98,000	51	0	chlorambucil, prednisone, propranol
10	F	71	CLL, stable	14,500	7	0	cylophosphamide, insulin
11	F	85	CLL, accelerating	10,500	1	0	prednisone
12	F	68	CLL, stable	10,500	83	0	cylophosphamide
13	M	55	CLL, stable	12,200	38	0	chlorambucil
14	F	56	CLL, stable	7,100	4	0	chlorambucil
15	M	—	CLL, stable	74,000	—	—	none
16	M	—	CLL, stable	16,000	—	—	chlorambucil
17	M	—	CLL, stable	46,000	—	—	none
18	M	73	HCL, stable	2,400	1	0	none
19	M	43	ALL, stable	8,000	2	0	none
20	F	23	ALL, stable	7,900	6	0	none
21	M	18	ALL, stable	5,000	1	0	6MP, MTX, vincristine, prednisone
22	M	31	ALL, accelerating	10,000	4	0	MTX
23	M	21	ALL, stable	2,600	0	0	none
24	M	24	ALL, stable	5,900	3	0	none
25	F	24	ANLL (M1 or M2), stable	7,200	3	0	none
26	F	41	ANLL (M1 or M2), stable	6,600	4	0	none
27	F	68	ANLL (M4), blast crisis	195,000	40	38	none
28	F	33	ANLL (M4), blast crisis	24,000	59	34	none
29	F	70	ANLL (M4), stable	2,500	3	0	none
30	M	69	ANLL (M4), blast crisis	40,000	70	54	none
31	M	26	ANLL (M3), stable	5,200	3	0	none
32	M	69	oligoblastic leukemia	7,700	18	9	prednisone
33	F	54	myelodysplasia	7,500	9	0	none
34	F	74	sideroblastic anemia	16,300	9	0	none
35	M	67	myelodysplasia	21,900	2	0	hydroxylurea
36	M	58	myelodysplasia	3,900	0	0	none
37	M	44	NHL, stable	6,000	3	0	none
38	M	72	NHL, stable	5,100	0	0	prednisone, cylophosphamide
39	F	62	NHL, stable	5,400	0	0	none
40	M	24	NHL, stable	4,100	0	0	none
41	F	43	NHL, stable	8,500	2	0	none
42	F	—	breast cancer	5,000	1	0	none
43	F	—	breast cancer	7,000	0	0	insulin
44	F	—	breast cancer	8,000	0	0	none
45	F	—	breast cancer	3,000	1	0	none
46	F	—	colon cancer	7,000	11	0	none
47	M	—	choriocarcinoma	6,000	5	0	none

[a]Abbreviations used: CML, chronic myelogenous leukemia; CMMoL, chronic monomyelocytic leukemia; CLL, chronic lymphocytic leukemia; HCL, hairy cell leukemia; ALL, acute lymphocytic leukemia; ANLL, acute nonlymphocytic leukemia (M1 + M2, acute myeloblastic leukemia; M3, acute promyelocytic leukemia; M4, acute myelomonocytic leukemia); NHL, non-Hodgkin's lymphoma; 6MP, 6-mercaptopurine; MTX, methotrexate.
[b]Not obtained at time of examination and blood sampling.

Chemicals

Sources of reagents and enzymes were as follows: gelatin, Tween-20, 4-chloro-1-naphthol, and electrophoresis grade Tris (Bio-Rad Laboratories, Richmond, CA); hydrogen peroxide, *E. coli* alkaline phosphatase, glucose-6-phosphate, and dithiothreitol (U.S. Biochemicals, Cleveland, OH); biotinylated donkey anti-rabbit immunoglobin (Cat. #RPN 1004) and a streptavidin, horseradish peroxidase conjugate (Cat. #RPN 1231) (Amersham Corp., Arlington Heights, IL); [3-^{14}C]HMG-CoA (57 mCi/mmol) and [5-^{3}H]mevalonolactone (24 Ci/mmol) (New England Nuclear, Boston, MA); and Per-

Downloaded from www.jlr.org by guest, on June 18, 2012

coll (Pharmacia, Piscataway, NJ). Cultured human leukocytes from the IM-9, NC-37, Daudi, Raji, Molt-4, CCRF-CEM and K562 cell lines were obtained from Meloy Laboratories (Springfield, VA). Polyclonal, monospecific anti-rat liver HMG-CoA reductase antiserum was kindly supplied by Dr. G. C. Ness. All other materials were from previously listed sources (28–31).

Solutions

Ten × Hank's Balanced Salts Solution contained 80 g/l NaCl, 4 g/l KCl, 10 g/l glucose, 600 mg/l KH_2PO_4, 475 mg/l Na_2HPO_4, and 170 mg/l phenol red (pH 7.4). Tris-buffered saline (TBS) contained 2.4 g/l Tris (pH 7.5) and 29.1 g/l NaCl. Seventy percent iso-osmotic Percoll contained 70 ml Percoll, 615 mg NaCl, and 30 ml of 0.15 M NaCl. Fifty-five percent iso-osmotic Percoll contained 55 ml Percoll, 0.48 g of NaCl, and 45 ml of 0.15 M NaCl. TEDK buffer contained 50 mM Tris (pH 7.5) 1.0 mM EDTA, 5.0 mM dithiothreitol, and 70 mM KCl.

Isolation of peripheral blood leukocytes

Blood samples (up to 40 ml) were obtained using tourniquet pressure from an antecubital vein with a 19-gauge needle and a 60-ml syringe. After removing the needle from the syringe, the blood was discharged into a 50-ml conical polystyrene centrifuge tube containing 1.0 ml of 250 mM EDTA (pH 7.4) and inverted several times. Care was taken to empty the syringe over 5 to 10 sec with the jet of blood coursing along the side of the tube. This was done to minimize hemolysis that may artifactually elevate leukocyte HMG-CoA reductase activity (32). Within 10 min of blood drawing, the sample was diluted with an equal volume of room temperature, Hank's Balance Salts Solution. Twenty-ml aliquots of the diluted blood sample were added to 50-ml plastic conical centrifuge tubes. For isolation of mononuclear leukocytes from healthy individuals or patients with either viral infections, nonhematologic malignancies, lymphocytic leukemia, monocytic leukemia, or non-Hodgkin's lymphoma, diluted blood samples were underlayered with 20 ml of room temperature Histopaque-1077 and centrifuged at 400 *g* for 40 min to sediment erythrocytes and granulocytes. For isolation of total leukocyte populations from normal individuals or patients with either preleukemia or myelocytic leukemia, diluted blood samples were underlayered with 20 ml of 70% iso-osmotic Percoll and centrifuged at 400 *g* for 40 min to sediment erythrocytes. After centrifugation, the "buffy coat" layer was isolated, washed twice in Hank's Balanced Salts Solution, once in 0.87% NH_4Cl to lyse contaminating erythrocytes, and once in TEDK buffer and then frozen in liquid N_2 for subsequent use.

Isolation of enriched populations of lymphocytes, monocytes, and granulocytes

Enriched populations of lymphocytes, monocytes and granulocytes from healthy subjects were isolated as described by Harwood, Schneider, and Stacpoole (28). Peripheral blood leukocytes from one unit of blood, isolated and concentrated by leukophoresis (30–50 ml leukocyte suspension/unit blood), were diluted to 120 ml with Hank's Balanced Salts Solution. Fifteen-ml aliquots of the diluted suspension were added to each of eight 50-ml conical centrifuge tubes. The suspension was underlayered first with 15 ml of 55% iso-osmotic Percoll and subsequently with 15 ml of 70% iso-osmotic Percoll. Discontinuous gradients were centrifuged for 40 min at 350 *g*. Mononuclear leukocytes were concentrated at the upper interface, while the granulocytes were concentrated at the lower interface. Erythrocytes were pelleted. The lower band (granulocytes) was removed and washed twice in RPMI-1640 tissue culture medium supplemented with 10% fetal bovine serum. The resulting cell pellet that contained greater than 95% granulocytes, was washed once in 0.87% NH_4Cl and once in TEDK and frozen in liquid N_2. The upper band (mononuclear cells) was removed, washed twice in RPMI-1640 tissue culture medium supplemented with 10% fetal bovine serum, and resuspended in 10 ml of the same medium. A 1.25-ml aliquot of the mononuclear cell population was applied to each of eight 24-ml (7 × 2.2 cm) iso-osmotic continuous Percoll gradients (d 1.010–1.160 g/ml) that had been previously prepared by centrifuging 55% Percoll in phosphate-buffered saline for 40 min at 21,000 *g*. After centrifugation at 1,000 *g* for 20 min, the upper band of leukocytes (migrating to d 1.060 g/ml and containing 70–89% monocytes) and the lower band of leukocytes (migrating to d 1.075 g/ml and containing greater than 99% lymphocytes) were each washed three times in RPMI-1640 supplemented with 10% fetal bovine serum and then twice in TEDK. The final cell pellets were resuspended in 0.1 ml of TEDK and frozen in liquid N_2 for subsequent use.

Growth and isolation of cultured leukocytes

Transformed but nonmalignant lymphoid cells of the IM-9 and NC-37 cell lines, and transformed malignant leukocytes of the Raji, Daudi, CCRF-CEM, Molt-IV, and K-562 cell lines were cultured in sterile RPMI-1640 medium containing 10% heat-inactivated fetal calf serum, 100 U/ml penicillin, 0.25 μg/ml fungizone, and 100 μg/ml streptomycin. Cell viability was greater than 90% as assessed by Trypan Blue dye exclusion. Leukocytes from late-log phase cultures were washed twice in 50 ml of TBS and frozen in liquid N_2 for subsequent use.

Isolation of leukocyte microsomes

Leukocyte microsomes were isolated as previously described (28). Frozen cell suspensions were incubated at room temperature until just thawed. Subsequent operations were at 0–5°C. Suspensions were homogenized 15 times with a ground-glass pestle in a 10-ml Potter-Elvehjem Tissue Homogenizer. Suspensions were transferred to a 50-ml Potter-Elvehjem Tissue Homogenizer

Downloaded from www.jlr.org by guest, on June 18, 2012

and homogenized 5 times with a motor-driven Teflon pestle. The homogenate was diluted to 8 ml with TEDK and centrifuged at 2,000 *g* for 15 min. The pellet was discarded and the resultant supernant liquid was centrifuged at 172,000 *g* for 90 min. After centrifugation, the microsomal pellet was resuspended in 0.16 ml TEDK per 10^8 cells homogenized.

Isolation and partial purification of human liver HMG-CoA reductase

Liver microsomal HMG-CoA reductase from a brain-dead human organ donor was solubilized from the microsomal membrane by limited proteolysis using a modification of the freeze-thaw, high glycerol method of Rogers, Panini, and Rudney (33). The solubilized enzyme was further purified by ammonium sulfate fractionation and subsequent heat fractionation at 65°C as previously described by Harwood, Greene, and Stacpoole (34). The resulting heat fraction of human liver HMG-CoA reductase had an initial specific activity of 376 pmol/min per mg protein and was stored frozen in liquid N_2 for subsequent use.

Measurement of human leukocyte HMG-CoA reductase activity

Leukocyte HMG-CoA reductase activity was measured as described by Harwood et al. (28), with the following exceptions. The specific activity of [3-^{14}C]HMG-CoA used for determining enzyme activity in freshly isolated leukocytes was 30 cpm/pmol, compared to a specific activity of 10 cpm/pmol used for assessing HMG-CoA reductase activity in cultured leukocytes (28, 35). EDTA, 68 mM, was included in all incubations to prevent conversion of mevalonate to phosphomevalonate. HMG-CoA reductase activity was measured in triplicate using 150 μg of microsomal protein for each sampling of freshly isolated leukocytes and in quadruplicate using 50 μg of microsomal protein for cultured leukocytes, and is expressed as pmoles of mevalonate formed per min of incubation at 37°C per mg of microsomal protein. Under the standard isolation conditions described above, HMG-CoA reductase is fully activated through dephosphorylation (28) and thiol-disulfide reduction (29, 31). No further increase in enzyme activity occurs upon treatment with additional dithiothreitol, or with either *E. coli* alkaline phosphatase or a crude preparation of rat liver phosphoprotein phosphatase (28, 31, 35).

Michaelis constants (K_m) for partially purified human liver HMG-CoA reductase and for the human leukocyte enzyme from healthy individuals and patients with hematologic malignancies were determined with respect to HMG-CoA at saturating concentrations of NADPH (3.4 mM) and variable concentrations of HMG-CoA between 1 and 40 μM by omitting [^{14}C]HMG-CoA from the substrate co-factor solution and by adding it directly to the assay mixture at the desired final concentrations. Michaelis constants with respect to NADPH were determined at saturating concentrations of HMG-CoA (67 μM) and variable concentrations of NADPH between 8 and 125 μM by omitting $NADP^+$ from the substrate co-factor solution and adding NADPH directly to the assay mixture of the desired final concentrations.

Measurement of HMG-CoA reductase protein concentration

Leukocyte microsomal HMG-CoA reductase protein concentration was measured as described by Harwood et al. (29). Briefly, portions of leukocyte microsomal preparations containing 1.5 μg of microsomal protein were applied to BA 85 nitrocellulose filters under vacuum using a Schleichter and Schuell Minifold-II slot blot apparatus. After sample application, the nitrocellulose sheet was removed from the blotting apparatus and incubated for 30 min at room temperature in 100 ml of TBS containing 3% gelatin. The nitrocellulose sheet was then incubated for 2 h at 45°C with 50 ml TBS containing 1% gelatin and 0.5 ml rabbit anti-rat HMG-CoA reductase antiserum. After two 15-min washes with 100 ml of TBS containing 0.05% Tween-20, and one 15-min wash with TBS, the nitrocellulose sheet was incubated for 60 min at room temperature with 40 ml of TBS containing 1% gelatin and 240 μl of biotinylated donkey anti-rabbit immunoglobulin (Amersham, Cat. #RPN 1004). After washing, as described above, the nitrocellulose sheet was incubated for 30 min at room temperature with 40 ml of TBS containing 1% gelatin and 80 μl of streptavidin-horseradish peroxidase conjugate (Amersham, Cat. #RPN 1231). After incubation, the nitrocellulose was again rinsed and washed as described above. After the final TBS wash, two solutions, one containing 40 mg of 4-chloro-1-naphthol in 10 ml of room temperature methanol and the other containing 50 μl of 30% H_2O_2 in 50 ml of room temperature TBS containing 1% gelatin, were mixed and immediately poured onto the nitrocellulose sheet. After a 15-min incubation at room temperature, the nitrocellulose sheet was washed with 200 ml of distilled water and air-dried. The intensity of color formation was quantitated by reflectance densitometry and was converted to μg immunoreactive protein by comparison to known human liver HMG-CoA reductase protein standards (29) included on each nitrocellulose sheet. Leukocyte microsomal HMG-CoA reductase protein concentration is expressed as μg immunoreactive protein per mg microsomal protein.

Measurement of microsomal protein concentration

Protein was determined by the method of Bradford (36), using bovine serum albumin as standard.

Downloaded from www.jlr.org by guest, on June 18, 2012

Lipid and lipoprotein analyses

Blood was obtained for serum lipid and lipoprotein determinations at the same time that samples were collected for leukocyte isolation. Serum total cholesterol and triglycerides were measured by standard automated procedures. High density lipoprotein cholesterol (HDL) was determined by heparin-manganese precipitation (37). Low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol concentrations were calculated by the method of Friedewald, Levy, and Fredrickson (38).

Blood leukocytes count and differential

Blood leukocyte counts were determined by automated analyses using a Coulter counter. Differential counts were determined by visual evaluation of Wright-stained blood smears. A minimum of 200 leukocytes were evaluated and were classified as either mature polymorphonuclear leukocytes, lymphocytes, monocytes, eosinophils, basophils, immature leukocytes (e.g., bands, metamyelocytes, myelocytes, promyelocytes) or blasts. Due to the ambiguity of assignments of "malignancy" to leukocytes in leukemia patient blood smears by visual inspection, no attempt was made to classify mature polymorphonuclear leukocytes, lymphocytes, or monocytes as either malignant or nonmalignant.

Statistical analysis

The unpaired Student's *t* test was used to determine statistical significance between mean values in the normal and leukemia/lymphoma populations.

RESULTS

Leukocyte HMG-CoA reductase activity is elevated in patients with hematologic malignancies

HMG-CoA reductase activity averaged 10.3 ± 0.8 pmol mevalonate formed/min per mg microsomal protein in freshly isolated mononuclear leukocytes from 31 healthy, normocholesterolemic subjects (**Table 2**), as reported previously (31). Enzyme activity was not substantially different in purified populations of lymphocytes (96% homogeneous), monocytes (81% homogeneous), and granulocytes (96% homogeneous) or in total leukocyte populations (Table 2). In contrast, leukocyte HMG-CoA reductase activity was elevated over threefold in patients with preleukemia, over fourfold in patients with leukemia, and over threefold in patients with non-Hodgkin's lymphoma (**Table 3**). The increase in enzyme activity that accompanied nonlymphocytic leukemia was approximately twofold greater than the increase in enzyme activity that accompanied the malignancies of lymphocytic origin (Table 3).

Approximately 50% of patients with preleukemia or acute leukemia had normal enzyme activity. In contrast, no patient with either chronic leukemia or lymphoma had an activity of HMG-CoA reductase that fell within two standard deviations of the normal mean (**Fig. 1**). One subject (patient 18) with hairy cell leukemia, a chronic leukemia presumably of lymphocytic origin (39), however, did exhibit normal enzyme activity.

Increased leukocyte HMG-CoA reductase activity was not due to nonspecific leukocyte stimulation or to the presence of malignancy per se, since increased enzyme activity was not observed in patients with viral infections (mononucleosis or influenza), or in patients with nonhematologic malignancies (breast cancer, colon cancer, choriocarcinoma) (Table 3).

Since the method of microsomal isolation fully activates the HMG-CoA reductase enzyme with respect to phosphorylation and thiol-disulfide reduction (28, 31), the increased leukocyte HMG-CoA reductase activity noted in Table 3 in patients with hematologic malignancies is not the result of enzyme activation through either increased dephosphorylation or increased thiol-disulfide reduction of that leukocyte enzyme. In studies in which peripheral blood leukocyte microsomes were incubated with either TEDK, TEDK containing 10 U *E. coli* alkaline phosphatase, or TEDK containing 20 mM dithiothreitol for 20 min at 37°C prior to measurement of HMG-CoA reductase activity, both the mononuclear leukocyte and total leukocyte enzymes isolated from normal subjects were fully active with respect to enzyme phosphorylation [9.75 ± 1.4 vs. 8.98 ± 1.9 (n = 11) and 14.7 ± 2.7 vs. 14.7 ± 2.7 (n = 4), respectively] and thiol-disulfide reduction [8.05 ± 0.5 vs. 8.35 ± 0.7 (n = 7) and 10.9 ± 1.7 vs. 11.9 ± 1.4 (n = 3), respectively].

TABLE 2. HMG-CoA reductase activity in populations of leukocytes from healthy subjects

Cell Type	HMG-CoA Reductase Activity
	pmol/min/mg
Total leukocyte populations (n = 3)	9.3 ± 1.0
Mononuclear leukocytes (n = 31)	10.3 ± 0.8
Lymphocytes (n = 8)	10.1 ± 0.8
Monocytes (n = 7)	13.0 ± 1.6
Granulocytes (n = 3)	8.8 ± 0.5

Forty-ml blood samples were obtained from 34 healthy, normocholesterolemic individuals at 8:00 AM after an overnight fast. Total leukocyte populations were isolated by density gradient centrifugation over 70% iso-osmotic Percoll while mononuclear leukocytes (77% lymphocytes:23% monocytes) were isolated by density gradient centrifugation over Histopague 1077, as described in Methods. Enriched populations of lymphocytes, monocytes, and granulocytes (96%, 81%, and 95% homogenous, respectively, as judged by Wright's and α-naphthyl acetate esterase staining) were isolated from 1 unit of blood from healthy blood donors as described in Methods. Leukocyte microsomes were isolated and microsomal HMG-CoA reductase activity was quantitated in triplicate for each individual. Data represent the mean ± SE of average HMG-CoA reductase activities for the indicated number of subjects.

Downloaded from www.jlr.org by guest, on June 18, 2012

TABLE 3. Leukocyte HMG-CoA reductase activity is elevated in patients with preleukemia, leukemia, or lymphoma but not in patients with viral infections or non-hematologic malignancies

Diagnosis	HMG-CoA Reductase Activity	Percent of Normal Activity	Fraction of Subjects in Normal Range
	pmol/min/mg	%	
Normal	10.3 ± 0.8	(100)	31/31
Preleukemia	36.9 ± 22[a]	349	2/5
Leukemia	43.9 ± 7[a]	426	7/31
ALL	26.4 ± 8[a]	256	3/6
ANLL	56.2 ± 23[a]	546	3/7
CLL	35.1 ± 5[a]	341	1/10[b]
CML	59.1 ± 13[a]	574	0/8
Non-Hodgkin's Lymphoma	36.0 ± 7[a]	350	0/5
Viral Infection	10.1 ± 1.2	98	6/6
Nonhematologic malignancies	9.3 ± 1.7	91	6/6

Blood volumes ranging between 10 and 40 ml were obtained from 31 healthy, normocholesterolemic individuals, 5 patients with preleukemia,[3] 6 patients with ALL, 7 patients with ANLL; (acute promyelocytic leukemia (FAB-M3), n = 1; acute myelogenous leukemia (FAB-M1 or M2), n = 2; acute monomyelocytic leukemia (FAB-M4), n = 4), 10 patients with CLL (CLL, n = 9; HCl, n = 1), 8 patients with CML (CML, n = 6; chronic monomyelocytic leukemia,[3] n = 2), 5 patients with non-Hodgkin's lymphoma (NHL), 6 patients with documented viral infections (4 with influenza, 2 with mononucleosis), and 6 patients with nonhematological malignancies (4 with breast cancer, 1 with colon cancer, and 1 with choriocarcinoma). For patients with leukemia and lymphoma, the volume of blood obtained was dependent on the patient's hematocrit and on the degree of elevation of the leukocyte count. For normal individuals, patients with viral infections, patients with non-hematological malignancies, and patients with ALL, CLL, CMMoL, and NHL, mononuclear leukocytes were isolated by density gradient centrifugation over Histopaque-1077, as described in Methods. For patients with preleukemia, ANLL, and CML, total leukocyte populations were isolated by density gradient centrifugation over 70% iso-osmotic Percoll. Leukocyte microsomes were isolated and microsomal HMG-CoA reductase was quantitated in triplicate for each individual as described in Methods. Data represent the mean ± SE of average HMG-CoA reductase activities for the indicated number of subjects. HMG-CoA reductase activity was considered to be within the normal range if it was within 2 standard deviations of the average HMG-CoA reductase activity for normal individuals (i.e., 1.5 to 19.1 pmol/min per mg).

[a] $P < 0.0001$.

[b] Of the 10 patients with CLL, only the patient with hairy cell leukemia had normal enzyme activity.

Differences in enzyme activity also were not the result of altered kinetic properties of the enzyme isolated from leukocytes of patients with hematologic malignancies. As shown in **Fig. 2**, the V_{max} for the microsomal enzyme isolated from the leukocytes of patient #9 (CLL) was 4.4-fold greater than that obtained from the microsomal enzyme isolated from the leukocytes of healthy individuals (45.9 pmol/min per mg vs. 10.4 pmol/min per mg). However, the Michaelis constant (K_m) for HMG-CoA obtained for the microsomal HMG-CoA reductase of patient #9 did not differ significantly from that obtained for the microsomal enzyme isolated from healthy individuals (12.8 μM; RS mixture) or from that obtained for a partially purified preparation of the human liver enzyme (heat fraction). The K_m value of 12.8 μM is also similar to the 10.3 μM K_m value obtained from the microsomal reductase of cultured human leukocytes of the IM-9 cell line (34). Thus differences in enzyme activity are not the result of differences in the kinetic properties of the enzyme.

The maximal velocity (V_{max}) values of 45.9 pmol/min per mg and 10.4 pmol/min per mg obtained from the kinetic analysis for the microsomal enzyme from patient #9 and for the microsomal enzyme from healthy individuals, respectively, did not differ significantly from the 46.2 pmol/min per mg and 10.3 pmol/min per mg, respectively, obtained by the standard methodology used to measure human leukocyte HMG-CoA reductase activity. Thus, the standard methology used in these studies measures enzyme activity at its maximum velocity, and minimizes the effects of altered substrate kinetic properties should they occur in a subpopulation of patients.

Neither total nor LDL cholesterol concentrations in the preleukemia, leukemia, or lymphoma patients studied differed significantly from plasma total or LDL cholesterol levels obtained for the normal population (**Table 4**). In contrast, however, plasma triglyceride and VLDL cholesterol levels were modestly, but significantly, elevated (Table 4), while plasma HDL concentrations were modestly, but significantly, reduced (Table 4). Similar increases in plasma triglyceride and VLDL cholesterol levels, with concomitant reduction of plasma HDL cholesterol levels, have been noted previously for both leukemia and lymphoma patients (40, 41) and are thought to be a consequence of decreased triglyceride clearance from the plasma, possibly as a result of altered lipoprotein

Downloaded from www.jlr.org by guest, on June 18, 2012

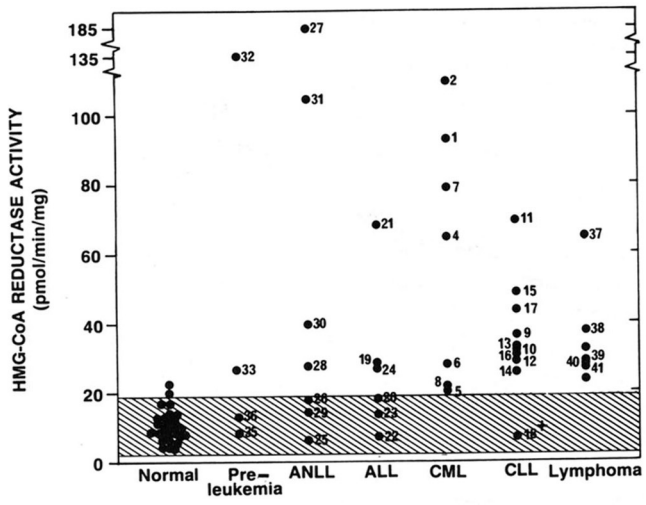

Fig. 1. Leukocyte HMG-CoA reductase activity for normal individuals and patients with leukemia or lymphoma. Peripheral blood mononuclear leukocytes were isolated and leukocyte microsomal HMG-CoA reductase activity was determined for the normal subjects, leukemia patients, and lymphoma patients described in Table 3 as outlined in Materials and Methods. Shown is the distribution of individual leukocyte HMG-CoA reductase activities for the 31 healthy volunteers, 31 leukemia patients, and 5 lymphoma patients described in Table 1 as a function of the classification of their hematologic malignancy. The shaded area represents two standard deviations above the mean HMG-CoA reductase activity for the normal population. The numbers next to individual data points represent the patient numbers listed in Table 1. Patient #18, who possessed hairy cell leukemia, a preplasma cell variant that is clinically distinct from CLL, is indicated by the + symbol.

lipase concentrations (40). The relationship, if any, between these differences in lipoprotein concentrations and increased leukocyte HMG-CoA reductase patients in leukemia and lymphoma are currently unknown.

Increased leukocyte HMG-CoA reductase activity in leukemia and non-Hodgkin's lymphoma is due to increases in both enzyme protein concentration and enzyme catalytic efficiency

The increased activity of HMG-CoA reductase in patients with leukemia or non-Hodgkin's lymphoma was due to increases in both enzyme catalytic efficiency and enzyme protein concentration (**Table 5**). Increases in enzyme catalytic efficiency closely paralleled the increases in enzyme activity noted in Table 3. HMG-CoA reductase catalytic efficiency was elevated approximately 2-fold in both acute and chronic lymphocytic leukemia and approximately 2.5-fold and 3.5-fold, respectively, in acute nonlymphocytic and chronic myelogenous leukemia (Table 5). HMG-CoA reductase protein concentration was also increased approximately 1.6-fold in leukemia. Thus, the over 4-fold increment in HMG-CoA reductase activity in leukemia was, in part (2.5-fold), the result of an increase in the catalytic efficiency of the enzyme molecule and, in part (1.6-fold), the result of an increase in the number of HMG-CoA reductase molecules. The individual differences between the enzyme catalytic efficiency values determined for leukocytes from healthy individuals and patients with leukemia are shown in **Fig. 3**.

Increased HMG-CoA reductase catalytic efficiency and enzyme protein concentration were also noted in patients with non-Hodgkin's lymphoma (Table 5). The increases in enzyme activity, enzyme protein concentration, and enzyme catalytic efficiency observed in non-Hodgkin's lymphoma resembled that noted in chronic lymphocytic leukemia, a disease of similar etiology (42, 43).

Leukocyte HMG-CoA reductase catalytic efficiency but not protein concentration is elevated in both transformed, nonmalignant, and malignant cultured leukocytes

To determine whether the increase in leukocyte HMG-CoA reductase activity is an integral aspect of the malignant phenotype, a consequence of cell growth, or a physiologic consequence of the malignancy, we asked whether increased enzyme catalytic efficiency and protein

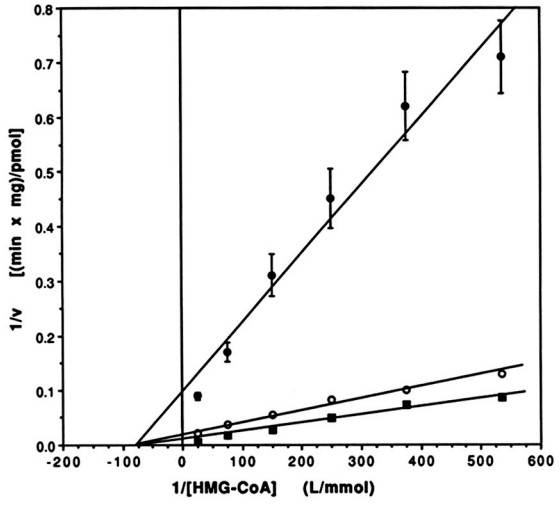

Fig. 2. Leukocyte microsomal HMG-CoA reductase from a patient with leukemia exhibits increased V_{max} with no change in K_m for HMG-CoA. Leukocyte microsomes (150 μg microsomal protein) from six healthy subjects, leukocyte microsomes (150 μg microsomal protein) from CLL patient #9, and partially purified human liver HMG-CoA reductase [heat fraction; 100 μg protein] were incubated for 30 min at 37°C in a final volume of 75 μl of TEDK buffer containing the indicated concentrations of [^{14}C]HMG-CoA and the remaining HMG-CoA reductase assay co-factors as described in Materials and Methods. For leukocyte microsomes from CLL patient #9 (○) and for partially purified human liver HMG-CoA reductase (heat fraction, ■), data are the average of duplicate measurements of HMG-CoA reductase activity. For the leukocyte microsomes from healthy subjects (●), data are the average (± SD) of the mean HMG-CoA reductase activities for the six microsomal samples. Shown is the reciprocal velocity for the HMG-CoA reductase catalyzed reaction as a function of reciprocal HMG-CoA concentration.

Downloaded from www.jlr.org by guest, on June 18, 2012

TABLE 4. Serum lipid and lipoprotein concentrations in normal individuals and patients with preleukemia, leukemia, and lymphoma

Diagnosis	N	Age	Total Cholesterol	VLDL Cholesterol	LDL Cholesterol	HDL Cholesterol	Triglycerides
		yr			*mg/dl*		
Normal	31	39 ± 3	194 ± 9	18 ± 1	129 ± 7	55 ± 2	92 ± 7
Preleukemia	5	65 ± 2[b]	191 ± 20	34 ± 7[b]	121 ± 25	40 ± 9[c]	170 ± 34[b]
Acute Leukemia	9	38 ± 5	191 ± 16	35 ± 5[a]	113 ± 13	43 ± 4[c]	176 ± 24[a]
Chronic Leukemia	12	67 ± 3[a]	186 ± 18	37 ± 6[a]	117 ± 14	43 ± 6[c]	185 ± 28[a]
Lymphoma	4	47 ± 7	186 ± 22	40 ± 7[a]	103 ± 14	43 ± 8[d]	198 ± 34[a]

Serum from 7 ml of blood was obtained from 31 healthy subjects, 5 patients with preleukemia, 9 patients with acute leukemia (6 with ALL, 3 with ANLL), 12 patients with chronic leukemia (7 with CLL, 1 with HCL, 2 with CML, 2 with CMMoL), and 4 patients with lymphoma after an 11–14 h overnight fast. Serum lipid and lipoprotein concentrations were measured as described in Methods. Data are the mean ± SE for the indicated number of individuals.

[a] $P < 0.0001$; [b] $P < 0.001$; [c] $P < 0.02$; [d] $P < 0.09$; all other values are not significantly different from the values obtained for the normal population.

concentration persisted in both transformed, nonmalignant and transformed, malignant cultured human leukocytes. As shown in **Table 6**, HMG-CoA reductase catalytic efficiency was increased between 12- and 33-fold, relative to peripheral blood mononuclear leukocytes, in all cell lines examined. However, HMG-CoA reductase protein concentration was not significantly increased in either the transformed, nonmalignant (IM-9, NC-37) or transformed, malignant (Daudi, Raji, Molt IV, CCRF-CEM, K-562) cell lines. The 18-fold increase in HMG-CoA reductase activity noted in cultured lymphoblasts (IM-9, NC-37 cell lines) that were transformed but not malignant (Table 6), is considerably greater than the 4- to 6-fold increase in HMG-CoA reductase activity that results from treatment of peripheral blood leukocytes with either phytohemagglutinin (PHA) (44, 45) or concanavalin A (ConA) (46), or the 3- to 4-fold increase in enzyme activity that results through release of cultured cells from mitotic arrest (47, 48), suggesting that the increase in HMG-CoA reductase catalytic efficiency is not a result of

TABLE 5. Increased leukocyte HMG-CoA reductase activity in leukemia and non-Hodgkin's lymphoma is due to increases in both enzyme protein concentration and enzyme catalytic efficiency

Diagnosis/Number	HMG-CoA Reductase Activity	HMG-CoA Reductase Protein Concentration	HMG-CoA Reductase Catalytic Efficiency
	pmol/min/mg	μg reductase/mg	pmol/min/mg reductase
Normal/31	10.3 ± 0.8	39 ± 4	313 ± 34
Leukemia/31	43.9 ± 7[a]	62 ± 6[b]	766 ± 95[a]
ALL/6	26.4 ± 8[a]	48 ± 8(NS)	640 ± 176[c]
ANLL/7	56.2 ± 23[a]	67 ± 15[c]	734 ± 170[a]
CLL/10	35.1 ± 5[a]	65 ± 11[c]	598 ± 124[b]
CML/8	59.1 ± 13[a]	65 ± 11[c]	1098 ± 245[a]
Non-Hodgkin's lymphoma/5	36.0 ± 7[a]	65 ± 11[c]	558 ± 32[c]

HMG-CoA reductase protein contained in the leukocyte microsomes isolated and used for HMG-CoA reductase activity measurement as described in Table 3 was quantitated by noncompetitive, solid phase enzyme immunoassay using polyclonal, monospecific anti-rat liver HMG-CoA reductase anti-serum, as described in Methods. Purified human liver HMG-CoA reductase standards (29) were included on each nitrocellulose sheet to minimize interassay variation and permit conversion of arbitrary reflectance units into μg immunoreactive protein. HMG-CoA reductase protein concentration was determined in triplicate for each individual. Data for HMG-CoA reductase activity is that of Table 3. Data for HMG-CoA reductase protein concentration is the mean ± SE of average HMG-CoA reductase protein concentrations for each of the indicated number of individuals. HMG-CoA reductase catalytic efficiency was calculated by dividing enzyme activity by enzyme protein concentration for each individual. Data for HMG-CoA reductase catalytic efficiency represent the mean ± SE of the calculated HMG-CoA reductase catalytic efficiencies for the indicated number of individuals.

[a] $P < 0.0001$; [b] $P < 0.005$; [c] $P < 0.01$; NS, not significant.

Downloaded from www.jlr.org by guest, on June 18, 2012

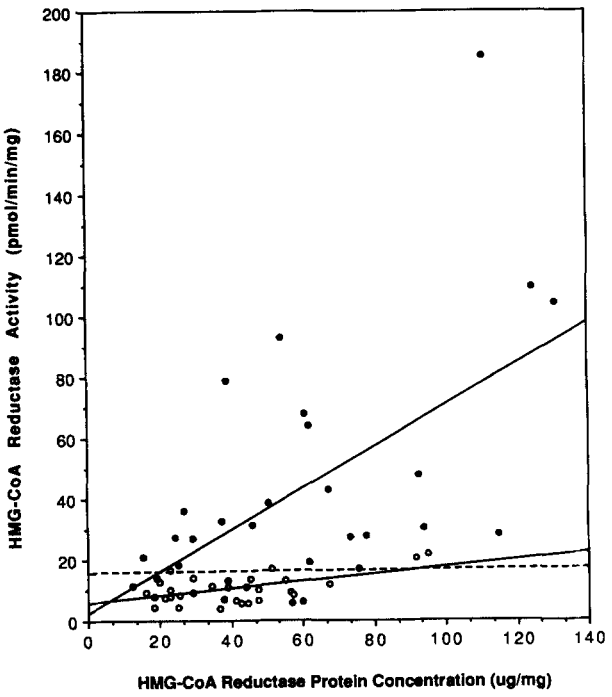

Fig. 3. Linear correlation between leukocyte HMG-CoA reductase activity and HMG-CoA reductase protein concentration in healthy individuals and patients with leukemia. Leukocyte HMG-CoA reductase activity and HMG-CoA reductase protein concentration were determined for normal subjects and leukemia patients described in Table 3, as outlined in Materials and Methods. Shown are the HMG-CoA reductase activities for the normal subjects (○; $r = 0.55$, $P < 0.001$) and leukemia patients (●; $r = 0.56$, $P < 0.002$) as a function of the HMG-CoA reductase protein concentration determined in the same microsomal preparation from which HMG-CoA reductase activity was determined. The dashed line represents two standard deviations above the mean HMG-CoA reductase activity for the normal population.

differences in enzyme activity in quiescent versus actively dividing cells. In addition, the approximately 2-fold increase in HMG-CoA reductase activity and catalytic efficiency in Raji and Daudi cells (malignant B-lymphocytes) relative to the transformed but nonmalignant lymphoblastic cell lines IM-9 and NC-37, all of which are rapidly dividing cell lines, further suggests that the difference in enzyme activity is not simply the result of differences between quiescent versus rapidly dividing cells. Finally, the increase in enzyme activity is not simply the result of exposure of lymphocytes to tissue culture growth medium since maintaining freshly isolated mononuclear leukocytes in tissue culture medium from up to 20 h resulted in only a slight (20%) and insignificant increase in HMG-CoA reductase activity (data not shown).

That both the transformed and nonmalignant cells and also the malignant leukocytes showed increases in HMG-CoA reductase catalytic efficiency supports the suggestion that the increase in HMG-CoA reductase catalytic efficiency is associated with malignant transformation rather than a consequence of the malignancy. Furthermore, the lack of increase in HMG-CoA reductase protein concentration in the cultured cell lines also supports the suggestion that the increase in enzyme protein levels may be secondary to physiological changes that occur during development of overt leukemia.

Biophasic elevation of leukocyte HMG-CoA reductase activity in acute nonlymphocytic leukemia

Historically, there is a high incidence of progression of preleukemia to acute nonlymphocytic leukemia (49). To further evaluate the role of increases in HMG-CoA reductase catalytic efficiency and enzyme protein concentration in the development of malignancy, we asked whether a qualitative difference in the mechanism responsible for the stimulation of HMG-CoA reductase activity could be noted between these two patient populations. As shown in **Table 7**, HMG-CoA reductase catalytic efficiency was elevated approximately 2.5-fold in both populations. However, whereas HMG-CoA reductase protein concentration was significantly increased by 1.7-fold in patients with acute nonlymphocytic leukemia, enzyme protein concentration was not raised in patients with preleukemia (Table 7). These data are consistent with the concept that increased enzyme catalytic efficiency is an early event associated with the conversion of normal leukocytes to "premalignant" leukocytes, whereas increased enzyme protein concentration is the result of a secondary response to the physiological changes that occur following development of malignancy. The lack of increase of HMG-CoA reductase protein concentration in cultured malignant leukocytes (Table 6), which are not subject to secondary physiological events resulting from the presence of malignancy, is consistent with this hypothesis.

DISCUSSION

The existence of an abnormally high level of HMG-CoA reductase activity in human leukemia has been previously inferred from increased rates of cholesterolgenesis from [^{14}C]acetate in leukocytes isolated from patients with a variety of leukemias (6, 7, 25). However, since normal granulocytes do not synthesize sterols (26, 27), and the majority of newly formed mevalonate in lymphocytes and monocytes is converted to nonsterol products (26, 27), the validity of these inferences remained questionable. To evaluate directly the effects of malignancy on the intrinsic properties of human leukocyte HMG-CoA reductase, we used recently developed techniques for measuring both HMG-CoA reductase activity (28) and HMG-CoA reductase protein concentration (29) and for calculating HMG-CoA reductase catalytic efficiency (specific activity) in microsomal fractions from either freshly isolated or cultured human leukocytes.

We found that HMG-CoA reductase activity was significantly elevated in freshly isolated leukocytes from pa-

Downloaded from www.jlr.org by guest, on June 18, 2012

TABLE 6. Increased HMG-CoA reductase activity in nonmalignant and malignant cultured human leukocytes is due to increased enzyme catalytic efficiency but not increased enzyme protein concentration

Cell Type	Cell Line	HMG-CoA Reductase Activity	HMG-CoA Reductase Protein Concentration	HMG-CoA Reductase Catalytic Efficiency
		pmol/min/mg	*μg reductase/mg*	*nmol/min/mg reductase*
Peripheral blood mononuclear leukocytes		10.3 ± 0.8	39 ± 4	0.31
Nonmalignant lymphoblasts	IM-9	189 ± 5	48 ± 3	3.9
Nonmalignant lymphoblasts	NC-37	179 ± 9	48 ± 3	3.8
Malignant B-lymphocytes	DAUDI	254 ± 12	40 ± 4	6.4
Malignant B-lymphocytes	RAJI	586 ± 40	78 ± 4	7.5
Malignant T-lymphocytes	Molt-IV	1170 ± 2	120 ± 9	10.1
Malignant T-lymphocytes	CCRF-CEM	170 ± 2	47 ± 4	3.7
Malignant myelocytes	K-562	336 ± 8	40 ± 3	8.3

Cultured, transformed but nonmalignant lymphoblasts of the IM-9 and NC-37 cell lines, cultured malignant lymphocytes of the Raji and Daudi cell lines, cultured malignant T-lymphocytes of the CCRF-CEM and Molt-4 cell lines, and cultured malignant myeloid cells of the K-562 cell line were isolated in late-log phase of growth by centrifugation at 400 *g* for 15 min. The culture medium was removed and the cell pellet was washed twice in phosphate-buffered saline and frozen in liquid N_2. Leukocyte microsomes were isolated and microsomal HMG-CoA reductase activity was determined as described in Methods. Data are the average of quadruplicate determinations of HMG-CoA reductase activity ± SE. HMG-CoA reductase protein in the cultured leukocyte microsomes was quantitated by noncompetitive, solid phase enzyme immunoassay using polyclonal, monospecific anti-rat liver HMG-CoA reductase antiserum, as described in Methods. Purified human liver HMG-CoA reductase standards (29) were included on each nitrocellulose sheet to minimize interassay variation and to permit conversion of arbitrary reflectance units to μg immunoreactive protein. HMG-CoA reductase protein concentration was determined in triplicate for each individual sample. Data for HMG-CoA reductase protein concentration is the mean ± SE of triplicate determinations. HMG-CoA reductase catalytic efficiency was calculated by dividing enzyme activity by enzyme protein concentration.

tients with a variety of hematological malignancies, including preleukemia, acute leukemia, chronic leukemia, and non-Hodgkin's lymphoma. The increase in enzyme activity was greater for nonlymphocytic leukemias than for malignancies of lymphocytic origin. In addition, whereas all patients with chronic leukemia or lymphoma had increased HMG-CoA reductase activity, only approximately 60% of the patients with acute leukemia or preleukemia exhibited increased enzyme activity.

It is not surprising that all patients studied with chronic leukemia exhibited elevated enzyme activity, since malignant cells circulate in these patients even during remission (50). Likewise, it is also predictable that only a portion of the patients with preleukemia and acute leukemia would demonstrate increased enzyme activity, since many were examined as outpatients with varying degrees of remission, in which relatively few malignant cells may circulate (50, 51). Increased enzyme activity in the malignant lymphomas, however, was an unexpected

TABLE 7. Biphasic elevation of leukocyte HMG-CoA reductase activity in acute nonlymphocytic leukemia

Diagnosis/Number	HMG-CoA Reductase Activity	HMG-CoA Reductase Protein Concentration	HMG-CoA Reductase Catalytic Efficiency
	pmol/min/mg	*ug reductase/mg*	*pmol/min/mg reductase*
Normal/31	10.3 ± 0.8	39 ± 4	313 ± 34
Preleukemia/5	36.9 ± 22[b]	38 ± 6(NS)	794 ± 348[b]
ANLL/7	56.2 ± 23[a]	67 ± 15[b]	734 ± 170[a]

HMG-CoA reductase protein concentration in the leukocyte microsomes isolated as described in Table 3 was quantitated by noncompetitive, solid phase enzyme immunoassay as described in Methods. Purified human liver HMG-CoA reductase standards (29) were included on each nitrocellulose sheet to minimize interassay variation and permit conversion of arbitrary reflectance units into μg immunoreactive protein. HMG-CoA reductase protein concentration was determined in triplicate for each individual. Data for HMG-CoA reductase protein concentration is the mean of the average HMG-CoA reductase protein concentration obtained for each of the indicated number of individuals ± SE. HMG-CoA reductase catalytic efficiency was calculated by dividing HMG-CoA reductase activity by HMG-CoA reductase protein concentration for each individual. Data for HMG-CoA reductase catalytic efficiency represent the mean ± SE of the calculated HMG-CoA reductase catalytic efficiencies for the indicated number of individuals.

[a] $P < 0.0001$; [b] $P < 0.01$; NS, not significant.

Downloaded from www.jlr.org by guest, on June 18, 2012

finding. However, since patients with lymphomas often progress through a leukemic phase (52), the possibility exists that biochemically abnormal cells with no apparent morphological abnormalities continually circulate in these patients. Recent reports have indicated the existence of circulating clonal B-cell populations in many, if not all, types of lymphomas (53, 54). In addition, the plasma lipid abnormalities (40, 41), which may be the result of reduced lipoprotein lipase activity (40), and the alterations in membrane fluidity (55–57; see below) noted in patients with leukemia are also found in patients with lymphomas (40, 41, 58), suggesting that similar disorders of lipid metabolism may occur in both classes of malignancy.

Increased leukocyte HMG-CoA reductase activity in hematological malignancies was not due to nonspecific leukocyte stimulation, since it was not observed in patients with viral infections. In addition, the increase in enzyme activity was not the result of increased rates of cell division since in freshly isolated and cultured malignant leukocytes the increase in enzyme activity relative to that of quiescent freshly isolated cells (17- to 114-fold) was substantially greater than that previously reported during induction of cell division in peripheral blood mononuclear leukocytes stimulated with lectins (44–46) or in cultured cells released from mitotic arrest (47, 48). Furthermore, increased enzyme activity was not due to the presence of cancer per se, since HMG-CoA reductase activity was normal in leukocytes from patients with nonhematological tumors, thus suggesting that the increase in enzymatic activity is not the result of production of a factor by tumor cells that stimulate leukocytes to increase their HMG-CoA reductase activity. Taken together, these data support the concept that increased HMG-CoA reductase activity may be a fundamental property of the malignant phenotype.

The results from these studies also indicate that the increase in leukocyte HMG-CoA reductase activity in malignancy is a complex phenomenon that is manifest through changes in both the catalytic efficiency (specific activity) and concentration of the enzyme. For patients with acute leukemia, chronic leukemia, or non-Hodgkin's lymphoma, changes in enzyme activity (4.3-fold) were predominately the result of an increase in enzyme catalytic efficiency (2.5-fold), with more modest increases in enzyme protein levels (1.6-fold). In contrast, increases in HMG-CoA reductase activity in patients with preleukemia (3.6-fold) were solely the result of changes in enzyme catalytic efficiency.

While the reasons for these differences are not readily apparent, several lines of evidence support the concept that increased enzyme catalytic efficiency is a fundamental property of the malignant phenotype, whereas increased enzyme protein concentration is a secondary consequence of nonspecific physiological changes that occur in an organism in the presence of malignancy. Increased enzyme catalytic efficiency persists following long-term culture of malignant cells, suggesting that the stimulus resulting in the increase in enzyme catalytic efficiency is intrinsic to the malignant cell. In contrast, increased HMG-CoA reductase protein levels are not seen in cultured malignant cells, suggesting that the elevated enzyme protein levels observed in freshly isolated leukocytes may be due to a stimulus extrinsic to the malignant cell. Consistent with this hypothesis is the observation that feedback regulation of HMG-CoA reductase synthesis by dietary cholesterol is lost in malignant tissues (2–5), but regained upon subsequent culture of the malignant cells (3, 19).

It has been reported that cholesterol synthesis is increased in livers of carcinogen-treated animals prior to development of frank hepatomas (5, 17). In our studies, patients with preleukemia exhibited increased HMG-CoA reductase activity due entirely to elevated enzyme catalytic efficiency, further suggesting that increased HMG-CoA reductase activity may be an early event in the development of malignancy. In contrast, patients with AML exhibited increased enzyme activity due to increases in both enzyme catalytic efficiency and enzyme protein concentration. Since a high proportion of patients with preleukemia progress to frank AML (49–51), it is possible that the increase in enzyme catalytic efficiency noted in preleukemia is a result of development of the "premalignant" state, whereas the increase in enzyme protein levels noted in AML reflects a secondary event that occurs concomitant to the progression from a premalignant state to a state of overt leukemia. Taken together, these findings are consistent with the hypothesis that a biphasic elevation of HMG-CoA reductase activity occurs during expression of malignancy, such that increased flux through preexisting enzyme molecules would occur early in the course of their disease, e.g., in the "premalignant" state. Subsequently, as the malignant process becomes overt, enzyme protein levels would increase and contribute further to the rise in enzyme activity. A more rigorous test of this premise, however, would require prospective evaluation of changes in leukocyte HMG-CoA reductase catalytic efficiency and enzyme protein concentration in patients progressing from a preleukemic to a frankly malignant state.

A variety of mechanisms exist whereby a cell can alter the catalytic efficiency of HMG-CoA reductase. These include covalent modification by phosphorylation (59, 60), enzyme thiol-disulfide reduction (61, 62), substrate-mediated allosteric activation (63, 64), and alterations in the fluidity of the microsomal membrane (65, 66). Although changes in the phosphorylation state of the enzyme have been shown to occur in a variety of malignancies (22, 23), the increases in enzyme activity noted in this report are not the result of changes in enzyme phosphorylation state. Nor are they the result of either

Downloaded from www.jlr.org by guest, on June 18, 2012

alterations in the thiol-disulfide status of the enzyme or substrate-mediated allosteric activation. The method of enzyme isolation fully activates the enzyme with respect to phosphorylation and thiol-disulfide reduction mechanisms of activity modulation and the concentrations of NADPH and HMG-CoA used in the assay fully activate the enzyme with regard to allosteric activation (63, 64). This latter point is exemplified by the observation that the activity of the microsomal enzyme isolated from the leukocytes of patient #9 (CLL) and that isolated from seven healthy subjects exhibited near identity with the V_{max} values calculated from the kinetic analysis represented in Fig. 2. Finally, since differences in HMG-CoA reductase activity persisted through kinetic analysis even though K_m values exhibited no differences between enzymes isolated from normal and malignant leukocytes, it is unlikely that the increased enzyme activity in leukemia and lymphoma is the result of differences in kinetic properties of the enzyme.

The activity of HMG-CoA reductase has also been shown to be regulated by the fluidity of the microsomal membrane, such that enzyme activity is increased with increasing membrane fluidity (65, 66). It is noteworthy that the cholesterol content of leukemic leukocytes is decreased approximately 50% relative to their normal counterparts (58, 67). As a result, the fluidity of the microsomal membrane, as judged by changes in both cholesterol to phospholipid ratios and a variety of order parameters, has been shown to be markedly increased in leukocytes isolated from patients with leukemia (55–58, 67). The decreased cholesterol content and increased microsomal membrane fluidity in leukemic leukocytes (55–58, 67) are both consistent with the increases we observed in HMG-CoA reductase catalytic efficiency, suggesting that increased HMG-CoA reductase catalytic efficiency in hematological malignancies may result from an alteration of microsomal membrane fluidity. In support of this suggestion is the observation that HMG-CoA reductase catalytic efficiency was elevated in all subjects with chronic hematologic malignancies, except for the patient with hairy cell leukemia, a chronic leukemia for which membrane fluidity has been reported to be normal (55). In addition, it is noteworthy that the cholesterol content and membrane fluidity of lymphoma cells are also decreased and increased, respectively (58), a finding consistent with the increase in HMG-CoA reductase activity we noted in patients with non-Hodgkin's lymphomas.

A variety of regulatory mechanisms also exist whereby a cell can affect increases in HMG-CoA reductase protein concentration. These include alterations in the rate of transcription (68, 69), translation (69, 70), mRNA half-life (69), and enzyme protein half-life (69, 71) that appear to be mediated in part by oxygenated forms of cholesterol (72, 73) and by nonsterol products of mevalonate metabolism (70, 74). For example, oxysterols, generated by oxidation of either newly formed cholesterol, cholesterol precursors, or internalized cholesterol (72), have been shown to lower HMG-CoA reductase protein concentration by reducing HMG-CoA reductase mRNA synthesis (69) and also by increasing the rate of HMG-CoA degradation (71). Nonsteroidal products of mevalonate metabolism reduce HMG-CoA reductase protein concentration by preventing HMG-CoA reductase mRNA translation (70) and also by increasing the rate of HMG-CoA reductase mRNA degradation (69). Alterations in any of these regulatory mechanisms in the malignant leukocyte could potentially result in an increase in HMG-CoA reductase protein concentration.

In addition, specific genetic mutations affecting regulatory regions of the genes or resultant mRNA molecules important in conferring normal transcriptional and translational regulation of HMG-CoA reductase production could affect such an increase in enzyme protein levels. In this regard, it is noteworthy that the chromosomal location of the HMG-CoA reductase gene (5q13–q23; 75, 76) is proximal to a region (5q13–q32) known to experience a high mutational frequency in a variety of hematologic malignancies (77–79). Whether this chromosomal abnormality is in part responsible for either a direct or indirect release of the HMG-CoA reductase gene from normal feedback regulatory mechanisms remains to be determined. However, the ability of cultured malignant cells to regain the capacity to control HMG-CoA reductase synthesis through feedback regulation (3, 19) suggests that a mutational event is unlikely to fully explain the increase in HMG-CoA reductase protein levels or the loss of feedback regulation in malignancy. Thus, alterations in metabolism that alter the concentrations of important regulatory molecules seems a more plausible explanation. Clearly, further examination of the mechanism whereby HMG-CoA reductase protein levels are increased in malignancy is necessary before a plausible explanation can be advanced. In addition, the consequences of the altered lipoprotein patterns in patients with hematologic malignancies on intracellular lipid metabolism and on HMG-CoA reductase protein levels also remain to be examined.

In summary, these studies indicate that increased HMG-CoA reductase activity in hematologic malignancies is due to abnormalities in at least two regulatory parameters that normally control mevalonate production: *1*) an increase in HMG-CoA reductase catalytic efficiency that may reflect alterations in membrane fluidity, and *2*) an increase in HMG-CoA reductase protein concentration that may occur subsequent to physiological changes that take place in response to the malignancy.

A number of studies have suggested that increased cholesterol/isoprenoid synthesis may be required for passage of cells through G_1 phase of the cell cycle (44, 80, 81). Since a number of products of mevalonate metabo-

Downloaded from www.jlr.org by guest, on June 18, 2012

lism have been implicated as potential growth regulators (69, 74, 80, 82–85), it is tempting to postulate that the increased HMG-CoA reductase activity and the ensuing increases in mevalonate, isoprenoid, and sterol synthesis may play a role in the rapid and uncontrolled growth rates of malignant cells. Indeed, recent reports have shown that the gene product of the *ras* oncogene, an oncogene whose presence has been noted in malignant and premalignant tissues (86), and whose overproduction has been linked to malignancy (87, 88), is isoprenylated (89, 90), and that this isoprenylation (farnesylation) may be important for its translocation to, and association with, the plasma membrane (90), its site of activity (88). These observations support the notion that the increase in mevalonate formation that results from increased HMG-CoA reductase activity may play a critical role in malignancy. Undoubtedly, the role of elevated HMG-CoA reductase activity in malignancy will become further clarified as the roles of isoprenoid metabolism and protein isoprenylation in cell growth regulation are further elucidated.

This work was supported by grants from the American Cancer Society (IN-62W, Project ACS-83-069), the National Cancer Institute (CA-40495), and the National Institutes of Health (HL-32550, DK-40439, and RR-00082). During the course of these studies, Dr. Harwood was the recipient of a New Investigator Research Award from the National Cancer Institute and Dr. Stacpoole was the recipient of a Research Career Development Award from the National Institutes of Health. We thank Drs. Richard Lottenberg and Warren Ross for their help in selecting appropriate patients for the study, Yvonne Greene and James Wasson for expert technical assistance, and Penny Moeller, Sheri Crouch, and Melody Riedy for secretarial assistance.

Manuscript received 5 October 1990 and in revised form 29 May 1991.

REFERENCES

1. Coleman, P. S., and B. B. Lavietes. 1981. Membrane cholesterol, tumorigenesis and the biochemical phenotype of neoplasia. *CRC Crit. Rev. Biochem.* **11:** 341–393.
2. Sabine, J. R. 1977. Cholesterol and cancer. *In* Cholesterol. Marcel Dekker Inc., New York. Chapter 11, 277–288.
3. Gregg, R. G., and P. A. Wilce. 1983. 3-Hydroxy-3-methylglutaryl coenzyme A reductase and cancer. *In* 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase. J. R. Sabine, editor. CRC Series in Enzyme Biology. CRC Press, Inc., Boca Raton, FL. Chapter 17, 245–257.
4. Chen, H. W., A. A. Kandutsch, and H. J. Heiniger. 1978. The role of cholesterol in malignancy. *Prog. Exp. Tumor Res.* **22:** 275–316.
5. Siperstein, M. D. 1970. Regulation of cholesterol biosynthesis in normal and malignant tissues. *Curr. Top. Cell. Regul.* **2:** 65–95.
6. Yachnin, S., R. A. Larson, and E. J. West. 1983. Rates of cholesterol biosynthesis are related to early differentiation in acute non-lymphocytic leukaemia cells. *Brit. J. Haematol.* **54:** 459–466.
7. Ho, Y. K., R. G. Smith, M. S. Brown, and J. L. Goldstein. 1978. Low-density lipoprotein (LDL) receptor activity in human acute myelogenous leukemia cells. *Blood.* **52:** 1099–1114.
8. Anderson, R. G. W., M. S. Brown, and J. L. Goldstein. 1981. Deficient internalization of receptor-bound low density lipoprotein in human carcinoma A-431 cells. *J. Cell. Biol.* **88:** 441–452.
9. Clayman, R. V., L. E. Bilhartz, D. K. Spady, L. M. Buja, and J. M. Dietschy. 1986. Low density lipoprotein receptor activity is lost in vivo in malignantly transformed renal tissue. *FEBS Lett.* **196:** 87–90.
10. Fabricant, M., and S. A. Broitman. 1990. Evidence for deficiency of LDL receptor on human colonic carcinoma cell lines. *Cancer Res.* **50:** 632–636.
11. Vitols, S., G. Gahrton, M. Björkholm, and C. Peterson. 1985. Hypocholesterolemia in malignancy due to elevated LDL receptor activity in tumor cells: evidence from studies in patients with leukemia. *Lancet.* **2:** 1150–1153.
12. Gottfried, E. L., 1967. Lipids of human leukocytes: relation to cell type. *J. Lipid Res.* **8:** 321–327.
13. Siperstein, M. D., and V. Fagan. 1964. Deletion of the cholesterol negative feedback system in liver tumors. *Cancer Res.* **24:** 1108–1115.
14. Erickson, S. K., A. D. Cooper, G. F. Barnard, C. M. Havel, J. A. Watson, K. R. Feingold, A. H. Moser, M. Hughes-Fulford, and M. D. Siperstein. 1988. Regulation of cholesterol metabolism in slow growing hepatoma in vivo. *Biochim. Biophys. Acta.* **960:** 131–138.
15. Danielwitz, M. D., G. A. Herrera, M. C. Kew, D. Mendelsohn, S. Barnes, C. B. Alexander, B. I. Hirschowitz, and J. G. Spenney. 1984. Autonomous cholesterol biosynthesis in murine hepatoma. A receptor defect with normal coated pits. *Cancer.* **54:** 1562–1568.
16. Barnard, G. F., S. K. Erickson, and A. D. Cooper. 1984. Lipoprotein metabolism by rat hepatomas. Studies on the etiology of defective dietary feedback inhibition of cholesterol synthesis. *J. Clin. Invest.* **74:** 173–184.
17. Horton, B. J., J. D. Horton, and J. R. Sabine. 1971. Metabolic controls in precancerous liver: defective control of cholesterol synthesis in rats fed N-2-fluorenyl acetamide. *Eur. J. Cancer.* **7:** 459–465.
18. Rodwell, V. W., J. L. Nordstrom, and J. J. Mitschelen. 1976. Regulation of HMG-CoA reductase. *Adv. Lipid Res.* **14:** 1–74.
19. Madden, E. A., E. J. Bishop, A. M. Fiskin, and G. Melnykovych. 1986. Possible role of cholesterol in the susceptibility of a human acute lymphoblastic leukemia cell line to dexamethasone. *Cancer Res.* **46:** 617–622.
20. Kandutsch, A. A., and R. L. Hancock. 1971. Regulation of the rate of sterol synthesis and the level of HMG-CoA reductase activity in mouse liver and hepatomas. *Cancer Res.* **31:** 1396–1401.
21. Brown, M. S., S. E. Dana, and M. D. Siperstein. 1974. Properties of HMG-CoA reductase solubilized from rat liver and hepatoma. *J. Biol. Chem.* **249:** 6585–6589.
22. Gregg, R. G., and P. A. Wilce. 1983. Reversible phosphorylation of HMG-CoA reductase in Morris hepatomas. *Biochem. Biophys. Res. Commun.* **114:** 473–478.
23. Feingold, K. R., M. H. Wiley, A. H. Moser, and M. D. Siperstein. 1983. Altered activation state of HMG-CoA reductase in liver tumors. *Arch. Biochem. Biophys.* **226:** 231–241.
24. Azrolan, N. I., and P. S. Coleman. 1989. A discoordinate increase in the cellular amount of HMG-CoA reductase results in the loss of rate-limiting control over cholesterolgenesis in a tumour cell-free system. *Biochem. J.* **258:** 421–425.
25. Heiniger, H. J., H. W. Chen, O. L. Applegate, Jr., L. P. Schacter, B. Z. Schacter, and P. N. Anderson. 1976. Elevated synthesis of cholesterol in human leukemia cells. *J. Mol. Med.* **1:** 109–116.
26. Shechter, I., A. M. Fogelman, and G. Popják. 1980. A defi-

ASBMB JOURNAL OF LIPID RESEARCH

Downloaded from www.jlr.org by guest, on June 18, 2012

ciency of mixed function oxidase activities in the cholesterol biosynthesis pathway of human granulocytes. *J. Lipid Res.* **21:** 277–283.
27. Fogelman, A. M., J. Seager, P. A. Edwards, M. Hokom, and G. Popják. 1977. Cholesterol biosynthesis in human lymphocytes, monocytes and granulocytes. *Biochem. Biophys. Res. Commun.* **76:** 167–173.
28. Harwood, H. J., Jr., M. Schneider, and P. W. Stacpoole. 1984. Measurement of human leukocyte microsomal HMG-CoA reductase activity. *J. Lipid Res.* **25:** 967–978.
29. Harwood, H. J., Jr., I. M. Alvarez, Y. J. Greene, G. C. Ness, and P. W. Stacpoole. 1987. Development of a noncompetitive, solid-phase, bridged biotin-avidin enzyme immunoassay for measurement of human leukocyte microsomal HMG-CoA reductase protein concentration. *J. Lipid Res.* **28:** 292–304.
30. Harwood, H. J., Jr., D. M. Bridge, and P. W. Stacpoole. 1987. In vivo regulation of human mononuclear leukocyte 3-hydroxy-3-methylglutaryl coenzyme A reductase. Studies in normal subjects. *J. Clin. Invest.* **79:** 1125–1132.
31. Stacpoole, P. W., D. M. Bridge, I. M. Alvarez, R. B. Goldberg, and H. J. Harwood, Jr. 1987. In vivo regulation of human mononuclear leukocyte 3-hydroxy-3-methylglutaryl coenzyme A reductase. Decreased enzyme catalytic efficiency in familial hypercholesterolemia. *J. Clin. Invest.* **80:** 1401–1408.
32. Harwood, H. J., Jr., D. M. Bridge, and P. W. Stacpoole. 1988. Erythrocyte contamination of leukocyte populations following density gradient centrifugation results in artificially high levels of human leukocyte HMG-CoA reductase activity. *Lipids.* **23:** 1154–1158.
33. Rogers, D. H., S. R. Panini, and H. Rudney. 1980. Rapid, high-yield purifications of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Anal. Biochem.* **101:** 107–111.
34. Harwood, H. J., Jr., Y. J. Greene, and P. W. Stacpoole. 1986. Inhibition of human leukocyte HMG-CoA reductase activity by ascorbate. An effect mediated by the free radical monodehydroascorbate. *J. Biol. Chem.* **261:** 7127–7135.
35. Harwood, H. J., Jr., M. Schneider, and P. W. Stacpoole. 1984. Regulation of human leukocyte microsomal hydroxymethylglutaryl CoA reductase activity by a phosphorylation and dephosphorylation mechanism. *Biochim. Biophys. Acta.* **805:** 245–251.
36. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.
37. National Heart and Blood Institute. 1974. Lipid Research Clinic's Manual I. Dept. of Health, Education and Welfare, National Institutes of Health, Bethesda, MD, Publication #75-628.74.
38. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1979. Estimation of the concentration of low density lipoprotein cholesterol in plasma without using the preparative ultracentrifuge. *Clin. Chem.* **7:** 397–402.
39. Yachnin, S., H. M. Golomb, E. J. West, and C. Saffold. 1983. Increased cholesterol biosynthesis in leukemia cells from patients with hairy cell leukemia. *Blood.* **61:** 50–60.
40. Spiegel, R. J., E. J. Schaefer, I. T. Magrath, and B. K. Edwards. 1982. Plasma lipid alterations in leukemia and lymphoma. *Am. J. Med.* **72:** 775–782.
41. Barclay, M., V. P. Skipski, O. Terebus-Kekish, E. M. Greene, R. J. Kaufman, and C. C. Stock. 1970. Effects of cancer upon high density and other lipoproteins. *Cancer Res.* **30:** 2420–2430.
42. Zacharski, L. R., and J. W. Linman. 1969. Chronic lymphocytic leukemia versus chronic lymphosarcoma cell leukemia. *Am. J. Med.* **47:** 75–81.
43. Leventhal, B. G., C. I. Civin, and G. Reaman. 1980. Markers in human lymphoid tumors. *In* Cancer Markers. Diagnostic and Developmental Significance S. Sell, editor. Humana Press, Clifton, NJ. Chapter 4, 89–114.
44. Chen, H. W., H. J. Heiniger, and A. A. Kandutsch. 1975. Relationship between sterol synthesis and DNA synthesis in phytohemagglutinin-stimulated mouse lymphocytes. *Proc. Natl. Acad. Sci. USA.* **72:** 1950–1954.
45. Chen, H. W., H. J. Heiniger, and A. A. Kandutsch. 1977. Stimulation of sterol and DNA synthesis in leukemic blood cells by low concentrations of phytohemagglutinin. *Exp. Cell. Res.* **109:** 253–262.
46. Chen, S. S. 1979. Enhanced sterol synthesis in concanavalin A-stimulated lymphocytes: correlation with phospholipid synthesis and DNA synthesis. *J. Cell Physiol.* **100:** 147–158.
47. Roussillon, S., M. Astruc, R. Defay, C. Tabacik, B. Descomps, and A. Crastes de Paulet. 1983. DNA and cholesterol biosynthesis in synchronized embryonic rat fibroblasts. I. Temporal relationships between HMG-CoA reductase activity, sterol biosynthesis and thymidine incorporation into DNA. *Biochim. Biophys. Act.* **763:** 1–10.
48. Quesney-Huneeus, V., M. H. Wiley, and M. D. Siperstein. 1979. Essential role for mevalonate synthesis in DNA replication. *Proc. Natl. Acad. Sci. USA.* **76:** 5056–5060.
49. Bagby, G. C., Jr. 1985. Definition of the preleukemic syndrome. *In* The Preleukemic Syndrome (Hemopoietic Dysplasia). Bagby, G. C. Jr., editor. CRC Press, Boca Raton, FL. Chapter 1, 3–18.
50. Klemperer, M. R, and M. A. Lichtman. 1978. The Leukemias. *In* Clinical Oncology for Medical Students and Physicians — a Multidisciplinary Approach. P. Rubin and R. F. Bakemeier, editors. American Cancer Society, Rochester, NY. Chapter 22, 245–258.
51. Champlin, R., and D. W. Golde. 1987. The Leukemias. *In* Harrison's Principles of Internal Medicine. E. Braunwald et al., editors. McGraw Hill, NY. 11th Ed. 1541–1550.
52. Cline, M. J. 1975. Lymphocytic lymphoma, Hodgkin's disease and other chronic lymphoproliferative diseases. *In* The White Cell. Harvard University Press, Cambridge, MA. Chapter 22, 425–456.
53. Smith, B. R., D. W. Weinberg, N. J. Robert, M. Towle, E. Luther, G. S. Pinkus, and K. A. Ault. 1984. Circulating monoclonal B lymphocytes in non-Hodgkin's lymphoma. *N. Engl. J. Med.* **311:** 1476–1481.
54. Weinberg, D. S., K. A. Ault, and G. S. Pinkus. 1988. Circulating malignant cells in non-Hodgkin's lymphoma: correlation with binding by peanut agglutinin. *Blood.* **72:** 698–704.
55. Van Blitterswijk, W. J., and J. Damen. 1985. Alterations in membrane lipid fluidity of tumor cells and in the plasma lipoprotein of tumor bearers, with special reference to leukemia. *In* Cholesterol Metabolism in Health and Disease: Studies in The Netherlands. A. C. Beynen et al., editors. Ponsen and Looijen Wageningen. 45–52.
56. Inbar, M., R. Goldman, L. Inbar, I. Bursuker, B. Goldman, G. Akstein, P. Segal, E. Ipp, and I. Ben-Bassat. 1977. Fluidity difference of membrane lipids in human normal and leukemia lymphocytes as controlled by serum components. *Cancer Res.* **37:** 3037–3041.
57. Deliconstantinos, G., S. Daefler, and G. R. Krueger. 1987. Cholesterol modulation of membrane fluidity and ectonucleotide triphosphatase activity in human normal and CLL lymphocytes. *Anticancer Res.* **7:** 347–352.
58. Shinitzky, M., and M. Inbar. 1974. Difference in microviscosity induced by different cholesterol levels in the surface membrane lipid layer of normal lymphocytes and malig-

Downloaded from www.jlr.org by guest, on June 18, 2012

nant lymphoma cells. *J. Mol. Biol.* **85:** 603–615.
59. Kennelly, P. J., and V. W. Rodwell. 1985. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by reversible phosphorylation-dephosphorylation. *J. Lipid Res.* **26:** 903–914.
60. Beg, Z. H., J. A. Stonik and H. B. Brewer, Jr. 1987. Modulation of the enzymic activity of HMG-CoA reductase by multiple kinase systems involving reversible phosphorylation: a review. *Metabolism.* **36:** 900–917.
61. Roitelman, J., and I. Shechter. 1984. Regulation of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase. Evidence for thio-dependent allosteric modulation of enzyme activity. *J. Biol. Chem.* **259:** 870–877.
62. Ness, G. C., M. J. McCreery, C. E. Sample, M. Smith, and L. C. Pendleton. 1985. Sulfhydryl/disulfide forms of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Biol. Chem.* **260:** 16395–16399.
63. Roitelman, J., and I. Shechter. 1984. Allosteric activation of rat liver microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase by nicotinamide adenine dinucleotides. *J. Biol. Chem.* **259:** 14029–14032.
64. Ness, G. C., S. J. Eales, L. C. Pendleton, and M. Smith. 1985. Activation of rat liver microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase by NADPH. Effects of dietary treatments. *J. Biol. Chem.* **260:** 12391–12393.
65. Mitropoulos, K. A., and S. Venkatesan. 1985. Membrane-mediated control of HMG-CoA reductase activity. *In* Regulation of HMG-CoA Reductase. B. Preiss, editor. Academic Press Inc., Orlando, FL. Chapter 1, 1–48.
66. Davis, P. J., and M. J. Poznansky. 1987. Modulation of HMG-CoA reductase by changes in microsomal cholesterol content or phospholipid composition. *Proc. Natl. Acad. Sci. USA.* **84:** 118–121.
67. Inbar, M., and M. Shinitzky. 1974. Cholesterol as a bioregulator in the development and inhibition of leukemia. *Proc. Natl. Acad. Sci. USA.* **71:** 4229–4231.
68. Clarke, C. F., A. M. Fogelman, and P. A. Edwards. 1984. Diurnal rhythm of rat liver mRNAs encoding HMG-CoA reductase. Correlation of functional and total mRNA levels with enzyme activity and protein. *J. Biol. Chem.* **259:** 10439–10447.
69. Goldstein, J. L., and M. S. Brown. 1990. Regulation of the mevalonate pathway. *Nature.* **343:** 425–430.
70. Peffley, D., and M. Sinensky. 1985. Regulation of HMG-CoA reductase synthesis by a nonsterol mevalonate-derived product. *J. Biol. Chem.* **260:** 9949–9952.
71. Jingami, H., M. S. Brown, J. L. Goldstein, R. G. W. Anderson, and K. L. Luskey. 1987. Partial deletion of membrane-bound domain of HMG-CoA reductase eliminates sterol-enhanced degradation and prevents formation of crystallized endoplasmic reticulum. *J. Biol. Chem.* **104:** 1693–1704.
72. Rudney, H., and R. C. Sexton. 1986. Regulation of cholesterol biosynthesis. *Annu. Rev. Nutr.* **6:** 245–272.
73. Taylor, F. R., S. E. Saucier, E. P. Shown, E. J. Parish, and A. A. Kandutsch. 1984. Correlation between oxysterol binding to a cytosolic binding protein and potency in the repression of HMG-CoA reductase. *J. Biol. Chem.* **259:** 12382–12387.
74. Brown, M. S., and J. L. Goldstein. 1980. Multivalent feedback regulation of HMG-CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *J. Lipid Res.* **21:** 505–517.
75. Mohandas, F., C. Heinzmann, R. S. Sparkes, J. Wasmuth, P. A. Edwards, and A. J. Lusis. 1986. Assignment of human HMG-CoA reductase gene to q12–q23 region of chromosome 5. *Somatic Cell Mol. Genet.* **12:** 89–94.
76. Lindgren, V., K. L. Luskey, D. W. Russel, and V. Franke. 1985. Human genes involved in cholesterol metabolism. Chromosomal mapping of the loci for the low density lipoprotein receptor and HMG-CoA reductase with cDNA probes. *Proc. Natl. Acad. Sci. USA.* **82:** 8567–8571.
77. Tunis, J. J. 1983. The chromosomal basis of human neoplasia. *Science.* **221:** 227–236.
78. Gyger, M., C. Perreault, R. Pichette, L. Forest, and P. Lussier. 1986. Interstitial deletion of the long arm of chromosomes 5 (5q-) in leukemia and other hematologic disorders: clinical and biological relevance of variable break point patterns. *Leuk. Res.* **10:** 9–15.
79. LeBeau, M. M., K. S. Albain, R. A. Larson, J. W. Vardiman, E. M. Davis, R. R. Blough, H. M. Golomb, and J. D. Rowley. 1986. Clinical and cytogenic correlators in 63 patients with therapy-related myelodysplastic syndromes and acute nonlymphocytic leukemia: further evidence for characteristic abnormalities of chromsomes #5 and #7. *J. Clin. Oncol.* **4:** 325–345.
80. Siperstein, M. D. 1984. Role of cholesterolgenesis and isoprenoid synthesis in DNA replication and cell growth. *J. Lipid Res.* **25:** 1462–1468.
81. Quesney-Huneeus, V., H. A. Galick, M. D. Siperstein, S. K. Erickson, T. A. Spencer, and J. A. Nelson. 1983. The dual role of mevalonate in the cell cycle. *J. Biol. Chem.* **258:** 378–385.
82. Hoffman, P. C., C. M. Richman, R. A. Larson, and S. Yachnin. 1983. Cholesterol and mevalonic acid are independent requirements for the in vitro profileration of human bone marrow granulocyte progenitor cells: studies using ML-236B. *Blood.* **61:** 667–671.
83. Beck, L. A., T. J. Hosick, and M. Sinensky. 1988. Incorporation of a product of mevalonic acid metabolism into proteins of CHO cell nuclei. *J. Cell. Biol.* **107:** 1307–1316.
84. Maltese, W. A., and K. M. Sheridan. 1987. Isoprenylated proteins in cultured cells: subcellular distribution and changes related to altered morphology and growth arrest induced by mevalonate deprivation. *J. Cell. Physiol.* **133:** 471–481.
85. Sepp-Lorensino, L., N. Azrolan, and P. S. Coleman. 1989. Cellular distribution of cholesterol-genesis-linked, phosphoisoprenylated proteins in proliferating cells. *FEBS Lett.* **245:** 110–116.
86. Kumar, R., S. Sukumar, and M. Barbacid. 1990. Activation of *ras* oncogenes preceding the onset of neoplasia. *Science.* **248:** 1101–1104.
87. Cooper, G. M., and M. A. Lane. 1984. Cellular transforming genes and oncogenesis. *Biochim. Biophys. Acta.* **738:** 9–20.
88. Santos, E., and A. R. Nebredia. 1989. Structural and functional properties of *ras* proteins. *FASEB J.* **3:** 2151–2163.
89. Schaber, M. D., M. B. Ohara, V. M. Garsky, S. D. Mosser, J. B. Bergstrom, S. L. Moores, M. S. Marshall, P. A. Friedman, R. A. F. Dixon, and J. B. Gibbs. 1990. Polyisoprenylation of *ras* in vitro by a farnesyl protein transferase. *J. Biol. Chem.* **265:** 14701–14704.
90. Hancock, J. F., A. I. Magee, J. E. Childs, and C. J. Marshall. 1989. All *ras* proteins are polyisoprenylated but only some are palmitoylated. *Cell.* **57:** 1167–1178.
91. Harwood, H. J., Jr., I. M. Alvarez, Y. J. Greene, W. D. Noyes, and P. W. Stacpoole. 1986. Increased HMG-CoA reductase protein concentration and catalytic efficiency in leukocytes from patients with leukemia or lymphoma. *Clin. Res.* **34:** 564A.

Downloaded from www.jlr.org by guest, on June 18, 2012