CHOLESTEROL BIOSYNTHESIS IN HUMAN LYMPHOCYTES, MONOCYTES, AND GRANULOCYTES

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Summary. Lymphocytes, monocytes and granulocytes were separated by counterflow centrifugation from the blood of normal individuals and were incubated in full serum medium or lipid-depleted medium. The monocytes incorporated about five times more [2-^{14}C]acetate into sterols than did the lymphocytes in full serum medium and approximately twenty times more than the lymphocytes in lipid-depleted medium. The granulocytes were unable to synthesize sterols from either [2-^{14}C]acetate or [2-^{14}C]mevalonate, but they were able to use these substrates for the synthesis of squalene and demonstrated approximately a two fold increase in the incorporation of [2-^{14}C]acetate (but not [2-^{14}C]mevalonate) into squalene when incubated in the lipid-depleted medium as compared to the full serum medium.

Our previous studies of leukocytes (1,2,3) were conducted on a mixed population of cells (approximately 65% granulocytes, 30% lymphocytes, and 5% monocytes). Having developed a technique which separates the leukocytes by the small differences in their sizes and to a lesser extent their densities, we now report on the ability of human lymphocytes, monocytes and granulocytes to incorporate acetate and mevalonate into the cholesterol biosynthetic pathway.

METHODS

Ten normal persons donated their blood. The mean (± standard error) of their cholesterol and triglyceride concentrations were 214±5 mg/dl, and 59±3 mg/dl, respectively. After a 12- to 14-hour fast, blood was drawn as previously described (2). However, instead of defibrinating the blood, clotting was prevented with heparin, 5 units/ml. The heparinized blood was then sedimented with plasmagel as described previously (2) and the leukocytes in the plasmagel supernatant were further separated by counterflow centrifugation; the tendency of particles to sediment in a centrifugal field was offset by the flow of liquid in the opposite direction. When the centrifugal and counterflow forces were exactly balanced, the particles remained suspended, neither sedimenting nor being flushed from the rotor. The separation was effected on the basis of cell size and to a lesser extent cell density. The plasmagel supernatant was injected into a Beckman Elutriator System which consisted of a Beckman JE-6 rotor in a Beckman J21B High Speed Refrigerated Centrifuge equipped with a strobe unit which illuminated the collection chamber within the rotor and allowed precise measurement of the rotor speed. Flow through the system was controlled with a Cole Parmer peristaltic pump which was turned off during the

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addition of the cells to the loading chamber of the system. The pump was turned on and the cells were loaded into the rotor with 200 ml of ice cold Krebs Ringer phosphate buffer, pH 7.4 containing 0.15 mM glucose and 1% bovine serum albumin at a flow rate of 7.8 ml/min. All the platelets and nearly all the red blood cells were washed out of the rotor at the conclusion of the loading phase. The rotor speed was kept constant at 2000 rpm and the temperature at 15° during the loading phase and the subsequent separation phase. The two phases together required about 1 h. After the cells were loaded into the rotor, fractions were collected into 40 ml siliconized centrifuge tubes packed in ice. Fraction 1 (lymphocyte fraction) was collected into three 40 ml tubes as the flow was increased from 10.0 to 12.5 ml/min. Fraction 2 (monocyte-rich fraction) was collected into four 40 ml tubes as the flow was increased from 14.0 to 17.5 ml/min. Fraction 3 (granulocyte fraction) represented the cells that remained in the rotor at a flow rate of 19.0 ml/min. The pump and centrifuge were stopped simultaneously and the cells remaining in the collecting chamber were transferred to a 40 ml siliconized tube. The three tubes of fraction 1 and the four tubes of fraction 2 were centrifuged at 400 x g for 10 min, the supernatants decanted, the cells resuspended and transferred into a single 40 ml tube for each fraction. After thorough mixing an aliquot was taken from each tube (Fractions 1,2,3) for cell counts and microscopic examination; the remaining cells were then distributed equally into 40 ml siliconized tubes, centrifuged at 400 x g for 10 min and transferred to 25 ml flasks with siliconized Pasteur pipets and were incubated at 37° under 95% O_2 - 5% CO_2 as previously described (2). The flasks contained Krebs-Ringer phosphate buffer, pH 7.4, fortified with 0.15 mM glucose, 1% bovine serum albumin, 100 units of penicillin/ml, 100 µg of streptromycin/ml, 0.33 mM Na acetate and 40% serum (full or lipid-depleted). The integrity and viability of the cells was determined at the end of the incubations; more than 95% of the cells were viable according to previously established criteria (2). Human AB-negative, complement-inactivated serum and solvent-extracted serum (lipid-depleted serum) were prepared as previously described (2) by the method of McFarlane (4). The cells were classified by their morphology, ability to ingest latex particles, and reaction with the α-naphthyl butyrate esterase (lipase) stain (5). The whole incubation mixture was saponified and the unsaponfiable petroleum-soluble radioactive products were separated on alumina columns into hydrocarbons and alcohols and were further analyzed by gas liquid radiochromatography and digitonin-precipitation as described previously (6,7).

RESULTS

The monocyte-rich fraction (Fraction 2) was by far the most active with respect to the incorporation of acetate into sterols (Table I). Moreover, when this fraction was incubated in the lipid-depleted medium there was more than a ten fold increase in the incorporation of acetate into sterols as compared to the same cells incubated in the full serum medium. The first experiment shown in Table I (Subject WC) demonstrates that the granulocytes (Fraction 3) were unable to synthesize sterols from acetate. The small amount of label detected in sterols in the granulocyte fraction in the other two experiments shown in Table I can be accounted for by the minor contamination of this fraction with monocytes. The granulocytes did synthesize some squalene from

TABLE I

The incorporation of [2-^{14}C]acetate into squalene and sterols by lymphocytes, monocytes and granulocytes

The leukocytes from three normal subjects were collected by plasmagel sedimentation and then separated into three fractions by counterflow centrifugation and incubated in duplicate in either full serum medium or lipid-depleted medium. After 5 h of incubation 48.78 μCi of [2-^{14}C]acetate (30 Ci/mol) were added. The incubation mixture was saponified 2 h later and analyzed as described previously (2).

Subject	Fraction No.	Composition of fractions			[2-^{14}C]acetate incorporated into squalene		sterols	
		lymphocytes	monocytes	granulocytes	in full serum medium	in lipid depleted medium	in full serum medium	in lipid depleted medium
		%	%	%	10^3dpm/h/10^8cells			
WC	1	99.8	0.2	0.0	1.04	4.62	1.56	6.82
	2	2.8	17.0	80.2	1.34	1.44	1.47	15.10
	3	0.0	0.0	100.0	0.39	0.72	0.00	0.00
LY	1	99.0	1.0	0.0	1.35	4.58	2.36	5.96
	2	8.3	55.3	36.8	3.20	4.09	6.32	64.70
	3	0.0	0.3	99.7	0.55	1.07	0.05	0.27
AF	1	98.0	2.0	0.0	1.34	5.10	2.09	9.48
	2	2.0	28.0	70.0	1.69	2.48	2.97	42.10
	3	0.0	0.2	99.8	0.63	1.05	0.05	0.38
Mean	1	98.9	1.1	0.0	1.24	4.77	2.00	7.42
±SE		±0.5	±0.5	0.0	±0.10	±0.17	±0.24	±1.1
Mean	2	4.4	33.4	62.3	2.08	2.67	3.59	40.60
±SE		±2.0	±11	±13	±0.57	±0.77	±1.4	±14
Mean	3	0.0	0.2	99.8	0.52	0.94	0.03	0.22
±SE		0.0	±0.1	±0.1	±0.07	±0.11	±0.02	±0.11

acetate and showed approximately a two fold increase in the incorporation of acetate into squalene when incubated in the lipid-depleted medium as compared to the full serum medium.

The experiments shown in Table II demonstrate that the granulocytes in the monocyte-rich fraction (Fraction 2) synthesized no more sterol than did the granulocytes in Fraction 3. Therefore, we can calculate the relative

TABLE II

The incorporation of [2-^{14}C]acetate into sterols by cells from the monocyte-rich fraction

The leukocytes from three normal subjects were collected by plasmagel sedimentation and then separated into three fractions as described in Table I. The monocyte-rich fraction (Fraction 2) was layered on Ficoll-Hypaque (density 1.080) and centrifuged at 400 x g for 40 minutes (8,9) and the monocyte-lymphocyte band (MLB) and the granulocytes at the bottom of the tube (pellet) were removed to separate tubes, washed three times in 25 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 0.15 mM glucose and 1% bovine serum albumin and then transferred to 25 ml flasks and incubated in duplicate in lipid-depleted medium. After 5 h of incubation 48.78 µCi of [2-^{14}C]acetate (30 Ci/mol) were added. The incubation mixture was saponfied 2 h later and analyzed as described previously (2).

Subject	Cell Fraction	Composition of fractions			[2-^{14}C]acetate incorporated into sterols
		lymphocytes	monocytes	granulocytes	
		%	%	%	10^3dpm/h/10^8cells
RT	MLB	25.0	74.2	0.8	110.00
	Pellet	0.0	0.4	99.6	0.25
DH	MLB	17.0	83.0	0.0	113.00
	Pellet	0.0	0.6	99.4	0.33
RS	MLB	14.4	85.6	0.0	107.00
	Pellet	0.2	1.4	98.4	1.36
Mean ±SE	MLB	19 ±3	81 ±3	0.3 ±0.3	110 ±2
Mean ±SE	Pellet	0.1 ±0.1	0.8 ±0.3	99 ±0.4	0.65 ±0.4

activities of the lymphocytes and monocytes with respect to the incorporation of acetate into sterols assuming that the granulocytes did not synthesize sterols. From the data in Table I we can calculate that the monocytes incorporated about five times more [2-^{14}C]acetate into sterols than did the lymphocytes in full serum medium and approximately twenty times more than the lymphocytes in lipid-depleted medium.

TABLE III

Utilization of [2-^{14}C]mevalonate by lymphocytes, monocytes, and granulocytes

The leukocytes from each of three normal subjects were collected by plasmagel sedimentation and then were separated into three fractions by counterflow centrifugation and incubated in duplicate in full serum medium. After 3 h of incubation 9.01 μCi of [2-^{14}C]mevalonate (13 Ci/mol) were added. The incubation mixture was saponified 2 h later and analyzed as described previously (2).

Subject	Fraction No.	Composition of fractions			[2-^{14}C]mevalonate incorporated into		
		lymphocytes	monocytes	granulocytes	non-sterol alcohols[a]	squalene	sterols
		%	%	%	10^3dpm/h/10^8cells		
GC	1	99.0	1.0	0.0	21.60	2.22	0.86
	2	14.0	45.0	41.0	55.80	63.10	8.42
	3	0.0	0.3	99.7	34.20	28.80	0.15
AM	1	96.0	4.0	0.0	16.60	3.57	1.40
	2	6.0	31.0	63.0	88.60	85.20	7.27
	3	0.0	0.5	99.5	62.70	45.00	0.19
PE	1	99.0	1.0	0.0	12.30	2.32	1.33
	2	10.8	41.8	48.0	51.10	78.00	10.30
	3	0.0	0.8	99.2	22.00	41.60	0.12
Mean ±SE	1	98.0 ±1.0	2.0 ±1.0	0.0 0.0	16.8 ±2.7	2.7 ±0.4	1.19 ±0.17
Mean ±SE	2	10.3 ±2.3	39.3 ±4.2	50.7 ±6.5	65.2 ±12	75.4 ±6.5	8.67 ±0.88
Mean ±SE	3	0.0 0.0	0.5 ±0.1	99.5 ±0.1	39.6 ±12	38.5 ±5.0	0.15 ±0.02

[a]These alcohols were mainly farnesol by gas liquid radiochromatography.

In contrast to the increased utilization of acetate for squalene synthesis by the granulocytes during the 5th to 7th hours of incubation in the lipid-depleted medium (Table I) and as previously shown for the mixed population of leukocytes (1,2,3) there was no increased utilization of mevalonate for the synthesis of farnesol or squalene and the granulocytes did not synthesize sterols from mevalonate either (data not shown). Therefore, we utilized the more convenient medium (full serum) and a more convenient time (a 3-hour

pre-incubation) for further studies on the incorporation of mevalonate (Table III). As shown previously (2) the leukocytes incorporated much more label into farnesol and squalene than into sterols. The monocyte-rich fraction was by far the most active with respect to the incorporation of label into sterols but in contrast to the experiments with acetate (Table I), the monocyte-rich fraction also incorporated much more [^{14}C]mevalonate into squalene than into sterols. The granulocytes synthesized more farnesol and squalene from mevalonate than did the lymphocytes but synthesized virtually no sterols.

DISCUSSION

The results of these experiments are in good agreement with our previous studies made on the whole leukocyte population (1,2,3). The greater capacity of each cell type to synthesize from mevalonate more squalene and farnesol than sterols would have been predicted. However, the relatively enormous capacity of the monocytes to synthesize sterols from either acetate or mevalonate and the inability of the granulocytes to synthesize sterols from either precursor were unexpected findings. The separation techniques employed in these experiments subjected each cell type to the same conditions and therefore the differences observed cannot be explained as artifacts of the separation process.

Recently, a number of investigators have used the lymphocyte-monocyte band obtained from the separation of whole blood by density-gradient centrifugation (10,11,12) and have referred to the cells that they obtained as "lymphocytes." In fact, this band would have contained on the average 24% monocytes with a range of 10-38% (9). Our data demonstrate the importance of recognizing the monocyte content in such experiments.

One might speculate whether there is a relationship between the short life span (1 to 2 days) of the granulocyte *in vivo* (13) and the long life span (3 months without division) of the monocyte (14) and their respective abilities to synthesize sterols and hence replace cholesterol lost from their membranes.

The granulocytes apparently retain the ability to regulate the flow of carbon through the 3-hydroxy-3-methylglutaryl Coenzyme A reductase step in the biosynthetic pathway to cholesterol, but have lost the ability to convert squalene into lanosterol.

There is strong evidence that in both lower animals and humans the tissue macrophages are derived largely from blood monocytes (15,16,17). Since the tissue macrophage participates in the formation of both tendon xanthomas and the atherosclerotic reaction (18), we believe that the human blood monocyte may serve as an excellent cell model in the study of atherosclerosis.

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