Differential regulation of the expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase, synthase, and low density lipoprotein receptor genes

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Abstract The ability of mitogenic stimulation of human T lymphocytes to alter the expression of genes involved in sterol metabolism was examined. Messenger RNA levels for 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, HMG-CoA synthase, and low density lipoprotein (LDL) receptor were quantified in resting and mitogen-stimulated T lymphocytes by nuclease protection assay. Mitogenic stimulation increased HMG-CoA synthase mRNA levels by 5-fold and LDL receptor by 4-fold when cells were cultured in lipoprotein-depleted medium whereas HMG-CoA reductase gene expression was not significantly increased. When cultures were supplemented with concentrations of low density lipoprotein sufficient to saturate LDL receptors, expression of all three genes was inhibited in resting lymphocytes, as effectively as was noted with fibroblasts. Similarly, LDL down-regulated gene expression in mitogen-activated lymphocytes so that mitogenic stimulation did not increase either HMG-CoA reductase or synthase mRNA levels, although LDL receptor gene expression was enhanced. These results indicate that expression of three of the genes involved in sterol metabolism is differentially regulated by LDL and mitogenic stimulation. Moreover, the increase in rates of endogenous sterol synthesis and the activity of HMG-CoA reductase in mitogen-stimulated T lymphocytes cannot be accounted for by increases in HMG-CoA reductase mRNA levels.—**Cuthbert, J. A., and P. E. Lipsky.** Differential regulation of the expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase, synthase, and low density lipoprotein receptor genes. *J. Lipid Res.* 1992. **33:** 1157–1163.

Supplementary key words mitogen-stimulated T lymphocytes • HMG-CoA reductase mRNA • HMG-CoA synthase mRNA • LDL receptor mRNA • low density lipoprotein

Entry of resting cells into the cell cycle is accompanied by a variety of biochemical events, including new membrane synthesis. The cholesterol required to synthesize new membranes may be provided either by the uptake of exogenous cholesterol-transporting lipoproteins or by endogenous synthesis of the necessary sterols. Both pathways are regulated by exogenous cholesterol and, in concert, maintain normal cellular cholesterol concentrations. The uptake of exogenous lipoprotein cholesterol is largely mediated by the low density lipoprotein (LDL) receptor, the expression of which is transcriptionally regulated by cellular sterols (1–3). The rate-limiting enzyme in endogenous sterol biosynthesis is 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the synthesis of mevalonate (4). The activity of HMG-CoA reductase is also regulated by changes in the exogenous cholesterol concentration (4). The effects of changes in the activation status of the cells on the expression of these genes, however, are less well defined.

In previous studies, we have stimulated peripheral blood mononuclear cells (PBMC) with mitogenic lectins and examined the effect of mitogen-induced cellular activation on expression of the LDL receptor gene (5). LDL receptor mRNA levels in freshly isolated PBMC are increased 6-fold by incubation in cholesterol-depleted medium (6). Mitogenic stimulation further increased LDL receptor mRNA levels by 3-fold over that induced by incubation in the absence of exogenous cholesterol (5). Furthermore, in PBMC incubated in the presence of saturating concentrations of LDL cholesterol, mitogenic stimulation increased LDL receptor mRNA levels by 9-fold (5). These findings suggest that mitogenic stimulation might regulate LDL receptor expression independently of LDL.

The current studies were undertaken to determine whether the endogenous pathway of sterol metabolism was also modulated by mitogenic stimulation, in a manner similar to the regulation of the LDL receptor pathway. Mitogenic stimulation increases endogenous sterol syn-

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low density lipoproteins; PBMC, peripheral blood mononuclear cells.

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thesis by 20-fold regardless of the presence of exogenous LDL cholesterol (7), suggesting that cell activation per se might provide a separate, regulatory signal for expression of gene products required for cholesterol biosynthesis. We therefore sought to ascertain whether there was coordinate regulation of the expression of the LDL receptor gene and the genes involved in the sterol biosynthetic pathway. The studies demonstrated that mRNA levels for the rate-limiting enzyme in cholesterol synthesis, HMG-CoA reductase, and for HMG-CoA synthase, the enzyme that catalyzes the immediately preceding step and mRNA for the LDL receptor, were differentially affected by mitogenic stimulation and exogenous LDL. Although coordinately regulated in resting PBMC and in fibroblasts, mitogenic stimulation affects mRNA levels for each of these genes differently.

MATERIALS AND METHODS

Measurement of mRNA levels by nuclease protection

Specific mRNA levels were quantitated by nuclease protection assay as previously described (5, 6). Briefly, plasmids containing cDNA inserts for human HMG-CoA reductase (pHRed-102) (8), HMG-CoA synthase (pHSyn-22) (9), γ-actin (pHFγA-1) (10), and the receptor for transferrin (pcD-TR1) (11) were kindly provided by Drs. Luskey (pHRed-102, The University of Texas Southwestern Medical Center, Dallas, TX), Brown, Goldstein, and Gil (pHSyn-22, The University of Texas Southwestern Medical Center, Dallas, TX), Gunning and Kedes (pHFγA-1, Stanford University, Palo Alto, CA), and Ruddle (pcD-TR1, Yale University, New Haven, CT). The HMG-CoA synthase probe was prepared from pHSyn-22 by digestion with *Hind*III and *Nsi*I, the resultant restriction fragment of 355 base pairs (bp) was subcloned into *Hind*III-*Pst*I-digested M13mp19 and sequenced by the dideoxy chain termination method (12). The fragment was 93% identical to hamster HMG-CoA synthase exon 3 (nucleotides 51 to 407) (9, 13), and was used to prepare single-stranded probes as detailed previously (6). Other single-stranded probes were prepared as follows. A 320-base pair (bp) *Hind*III-*Taq*I fragment of pHRed-102, encompassing nucleotides 1558–1878 from the coding region of human HMG-CoA reductase, corresponding to exon 8 and parts of exons 7 and 9 in the hamster HMG-CoA reductase gene (14), was subcloned into *Hind*III-*Acc*I-digested M13mp19; a 496 bp *Taq*I fragment of pHFγA-1, containing nucleotides 1331–1827 of γ-actin (10) was subcloned into the *Acc*I site of M13mp19; and a 237 bp *Kpn*I fragment of pcD-TR1, containing nucleotides 1567 to 1798 of the transferrin receptor, was subcloned into the *Kpn*I site of M13mp19. The full lengths of the probes, including vector sequences, were: γ-actin = 569 nucleotides (nt), HMG-CoA synthase = 414 nt; HMG-CoA reductase = 394 nt; transferrin receptor = 304 nt.

Single-stranded ^{32}P-labeled probes were synthesized with an oligonucleotide primer in the presence of 0.5 μM [α-^{32}P]dCTP (~3000 Ci/mmol, ICN Chemical and Radioisotope Division, Irvine, CA), dCTP (0.75 μM for HMG-CoA reductase, 3 μM for HMG-CoA synthase, 30 μM for transferrin receptor, and 270 μM for γ-actin probes), 0.1 mM dATP, dTTP, and dGTP; and the Klenow fragment of *Escherichia coli* DNA polymerase as detailed (15). The extended product was digested with *Hind*III (Boehringer-Mannheim Biochemicals) and the resultant ^{32}P-labeled probe was purified by 7 M urea–6% polyacrylamide gel electrophoresis, electroelution, and ethanol precipitation.

Total RNA was isolated as described previously (6) and 10–20 μg was hybridized with multiple ^{32}P-labeled probes simultaneously at 45°C overnight as detailed (6), and then digested with 5–10 units of mung bean nuclease (Bethesda Research Laboratories) in a buffer containing 50 mM sodium chloride, 10 mM sodium acetate, pH 4.6, 1 mM zinc chloride, 1 mM β-mercaptoethanol, and 0.001% Triton X-100. After precipitation with carrier salmon sperm DNA (1 μg), samples were analyzed on 7 M urea–6% polyacrylamide gels with ^{32}P-labeled *Msp*I fragments of pBR322 (New England Biolabs, Beverly, MA) as size standards.

After electrophoresis, the gels were fixed and dried before being exposed to Kodak XAR-5 film for 12–24 h at room temperature. Following exposure, radiolabeled bands were identified, excised, and the ^{32}P-labeled cDNA content was quantified by liquid scintillation spectroscopy. The ^{32}P-labeled cDNA content of identically sized bands from lanes containing no RNA was used as a measurement of nonspecific background and was subtracted from all values. The HMG-CoA synthase cDNA probe corresponded to exon 3, which is not differentially spliced (9); therefore all HMG-CoA synthase mRNA species were measured by this technique, regardless of alternative splicing and polyadenylation sites. Similarly, the HMG-CoA reductase cDNA probe corresponded to the middle of the coding region and consequently measured all HMG-CoA reductase mRNAs, regardless of transcription initiation and polyadenylation signals. Comparisons within an experiment in which the same ^{32}P-labeled cDNA probe was utilized were used to determine changes in abundance of mRNA.

In experiments where sterols were added to some cultures, results were expressed as relative mRNA levels calculated using the amount of actin or transferrin receptor mRNA in the corresponding samples (either unstimulated or mitogen-stimulated) without sterols to correct for procedural losses. In these studies, experimental results are expressed relative to those of control cells normalized so that they contained 100 arbitrary units of mRNA.

Cell isolation and culture

PBMC were isolated from anticoagulated venous blood of healthy adults as previously described (5, 6). For some experiments, T cell-enriched populations (>95% rosette positive) were obtained by rosetting with neuraminidase-treated sheep red blood cells followed by passing rosette-positive cells over a nylon wool column as detailed (16). Cells were cultured in RPMI 1640 medium supplemented with 1% lipoprotein-poor plasma (d > 1.230 g/ml) prepared as described previously (17). Phytohemagglutinin (Wellcome Reagents Ltd., Research Triangle Park, NC) at the previously determined optimal concentration (0.5 μg/ml) was used as the mitogenic stimulus for all experiments. Where indicated, cultures were supplemented with various concentrations of LDL prepared as described (17).

Normal human diploid fibroblasts were obtained from Dr. J. R. Smith (Baylor College of Medicine, Houston, TX) and maintained in basal medium, a 4:1 mixture of Dulbecco's modified Eagle's medium:medium 199, supplemented with 10% defined supplemented calf serum (Hyclone Laboratories, Logan, UT). The medium was changed to basal medium supplemented with 10% lipoprotein-poor plasma with additions as indicated in the individual experiments 2–3 days after plating and the cultures were incubated for a further 24 h before harvesting cells for RNA isolation. At the time of harvest, cells were in logarithmic growth phase.

Statistics

Statistical analyses were performed using Student's two-tailed *t* test.

RESULTS

Mitogenic stimulation increases both reductase and synthase mRNA levels

In the initial experiments, the effect of mitogenic stimulation on HMG-CoA reductase and HMG-CoA synthase mRNA levels in PBMC cultured in lipoprotein-depleted medium was determined. As shown in **Fig. 1,** the mitogenic lectin phytohemagglutinin induced an increase in mRNA levels for both HMG-CoA reductase and HMG-CoA synthase, as well as for γ-actin and the receptor for transferrin. Mitogenic stimulation increased mRNA levels for γ-actin by 2.6 ± 0.2 fold (mean ± SEM for 15 experiments). Of the sterol regulatory genes, the magnitude of the increase was greatest for HMG-CoA synthase, with a mean induction of 5.1 ± 0.8-fold (mean ± SEM, n = 9; $P < 0.001$). In contrast, the increase in HMG-CoA reductase mRNA levels with mitogenic stimulation was minimal and not statistically significant (1.9 ± 0.5-fold, n = 13; $P > 0.10$). In only one individual of 13 tested were HMG-CoA reductase mRNA levels increased to a greater degree than mRNA levels for the "housekeeping" gene γ-actin. There was no increase in HMG-CoA

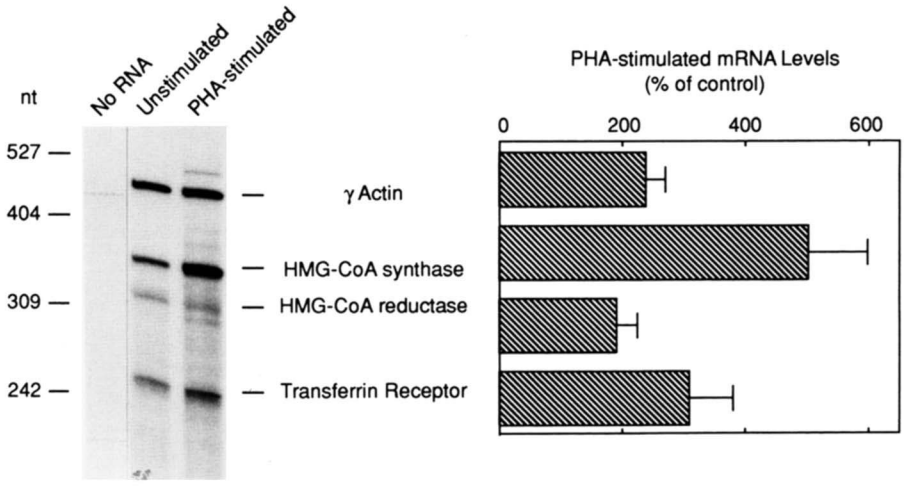

Fig. 1. Mitogenic activation increases both reductase and synthase mRNA levels. PBMC were incubated for 24 h in lipoprotein-deficient medium with or without phytohemagglutinin (PHA). Total RNA was isolated and 20 μg was hybridized with ^{32}P-labeled probes. The protected bands were identified by comparison to a ^{32}P-labeled *Msp*I-digest of pBR322 separated by electrophoresis in adjacent lanes (γ-actin probe = 569 nt; actin-protected band = 496 nt; HMG-CoA synthase probe = 414 nt; HMG-CoA synthase-protected band = 355 nt; HMG-CoA reductase probe = 394 nt; HMG-CoA reductase-protected band = 320 nt; transferrin receptor probe = 304 nt; transferrin receptor-protected band = 237 nt). Left panel: Autoradiograph depicting typical effects of mitogenic stimulation on mRNA levels. Right panel: Quantitative results shown as percent change resulting from mitogenic stimulation ± SEM for 9–15 experiments.

reductase mRNA levels in PBMC with mitogenic stimulation when results were normalized for γ-actin mRNA levels (0.6 ± 0.1-fold, n = 13). Similar results were obtained when cell populations enriched for mitogen-responsive T lymphocytes were examined (see below). Maintenance of steady-state mRNA levels in mitogen-stimulated PBMC required ongoing protein and RNA synthesis. Both actinomycin D and cycloheximide decreased HMG-CoA reductase and synthase mRNA levels in mitogen-stimulated PBMC when added 2 h (actinomycin D) and 6 h (cycloheximide) before isolation of RNA (**Fig. 2**).

Sterol regulation of HMG-CoA reductase and synthase mRNA levels in human T lymphocytes and fibroblasts

The effect of exogenous sterols on reductase and synthase mRNA levels was then determined in T cell-enriched populations. LDL cholesterol decreased both mRNA species (**Table 1**). Regulation of HMG-CoA reductase and HMG-CoA synthase mRNA levels in proliferating human diploid fibroblasts was similar to that observed in T cells. LDL receptor mRNA levels were quantitated in the same samples; exogenous LDL down-regulated mRNA in resting T cells, fibroblasts, and mitogen-activated T cells (Table 1). Differences in the efficiency of sterol regulation of HMG-CoA reductase, HMG-CoA synthase, and LDL receptor mRNA in unstimulated and mitogen-stimulated T cells were apparent when the effect of mitogenic stimulation in the presence of LDL was calculated (**Fig. 3**). Whereas phytohemagglutinin increased all three sterol-regulated mRNA levels to varying degrees when cells were cultured in lipoprotein-depleted medium, only LDL receptor mRNA levels were induced by mitogenic stimulation when cells were cultured in medium containing saturating concentrations of LDL cholesterol. Similar results were obtained in experiments with PBMC. Thus, LDL receptor mRNA levels were increased 8.6 ± 1.6-fold (n = 6; $P < 0.01$) by mitogenic stimulation in PBMC cultured in LDL-supplemented medium (50 μg LDL cholesterol/ml) whereas the increase was 4.6 ± 1.3-fold ($P < 0.05$ compared with unstimulated; $P < 0.05$ compared with PHA-stimulated in LDL-supplemented medium) when parallel cultures of PBMC were incubated in lipoprotein-depleted medium. In contrast, HMG-CoA reductase mRNA levels were increased only 1.5 ± 0.2-fold (n = 5; $P > 0.05$) by mitogenic stimulation of PBMC when LDL (50 μg cholesterol/ml) was added. These results suggest that down-regulation of HMG-CoA reductase and synthase but not LDL receptor gene expression by sterols is able to override the effects of mitogenic stimulation.

TABLE 1. Sterol regulation of mRNA levels in human T lymphocytes and fibroblasts

Addition	Specific mRNA		
	HMG-CoA Reductase	HMG-CoA Synthase	LDL Receptor
	% inhibition		
Unstimulated T cells			
LDL 5 μg cholesterol/ml	26	11	71
LDL 50 μg cholesterol/ml	70	72	84
PHA-stimulated T cells			
LDL 5 μg cholesterol/ml	51	47	47
LDL 50 μg cholesterol/ml	93	87	65
Human fibroblasts			
LDL 50 μg cholesterol/ml	79	87	86

Cells were cultured in medium supplemented with lipoprotein-poor plasma and with varying concentrations of LDL as indicated for 24 h before isolation of total RNA.

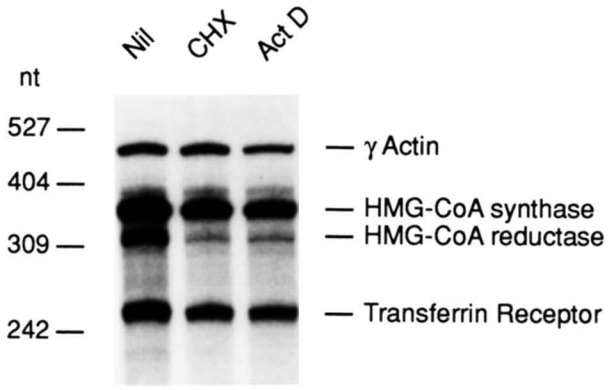

Fig. 2. Requirement for RNA and protein synthesis for maintenance of mRNA levels. PBMC were incubated in lipoprotein-deficient medium with phytohemagglutinin (PHA). Cycloheximide (CHX, 10 μg/ml) was added after 18 h (lane 2) and RNA was isolated after 24 h (lanes 1 and 2) or actinomycin D (Act D, 10 μg/ml) was added after 24 h and RNA was isolated after 26 h incubation (lane 3).

DISCUSSION

The current study compares changes in the expression of three genes that are involved in sterol metabolism, HMG-CoA reductase, HMG-CoA synthase, and LDL receptor. Mitogenic stimulation increased mRNA levels of all three genes when T cells were cultured in lipoprotein-depleted medium. However, the increase in HMG-CoA reductase expression was modest and not significantly different from that of γ-actin. Addition of exogenous LDL cholesterol down-regulated both HMG-CoA reductase and synthase mRNA levels, such that mitogenic stimulation no longer generated an increase in gene expression. In contrast, mitogenic stimulation increases LDL receptor mRNA levels even when saturating concentrations of LDL are present (5). These findings indicate that there are significant differences in the regulation of three of the genes involved in sterol metabolism. Although they are regu-

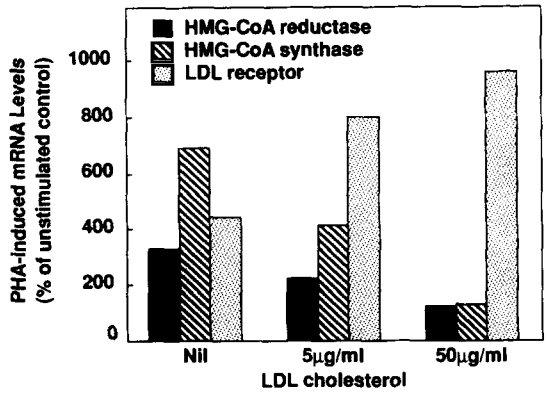

Fig. 3. Effect of sterols on mitogen-induced regulation of mRNA levels in human T cells. T cell-enriched populations were prepared and RNA was isolated after a 24-h incubation in lipoprotein-deficient medium with or without phytohemagglutinin and LDL (5 or 50 μg cholesterol/ml). mRNA-protected bands were quantified by scintillation spectroscopy and the relative mRNA levels in phytohemagglutinin-activated T cells were calculated by comparison with unstimulated T cells for each concentration of ambient sterol. Each point represents the increase in mRNA levels induced by mitogenic stimulation, calculated by comparing the levels in parallel unstimulated cultures, containing the identical concentrations of LDL.

lated in a coordinate fashion in resting T lymphocytes and in fibroblasts, after mitogenic stimulation of T lymphocytes significant differences emerge. Thus, HMG-CoA synthase and LDL receptor mRNAs are stimulated to an increase above that of housekeeping genes such as γ-actin, whereas in the presence of saturating concentrations of LDL, only LDL receptor mRNA is increased by mitogenic stimulation.

Mitogenic stimulation of human T cells increases the expression of many genes (18). For example, we have previously demonstrated that LDL receptor gene expression is transcriptionally up-regulated following T cell activation (5). The increase in LDL receptor mRNA levels cannot be ascribed to depletion of intracellular sterols since esterification of newly synthesized cholesterol continues unchanged. The mitogen-mediated augmentation in LDL receptor mRNA levels is observed in cells induced by incubation in lipoprotein-deficient medium as well as in cells incubated with saturating concentrations of LDL cholesterol. Thus, the signal provided by mitogenic stimulation apparently up-regulates LDL receptor gene expression independently of sterol down-regulation (5).

The effects of mitogenic stimulation on HMG-CoA reductase and synthase are in contrast to the findings reported previously for LDL receptor gene expression. Although mitogenic stimulation increased both HMG-CoA reductase and synthase mRNA levels when cells were cultured in lipoprotein-depleted medium, there were no such increases when saturating concentrations of LDL cholesterol supplemented the cultures. The present results conflict with the recently reported finding that there was a substantial increase (30-fold) in HMG-CoA reductase mRNA levels in mitogen-stimulated T lymphocytes cultured in medium supplemented with lipoproteins from fetal bovine serum (19). In the current studies, in contrast, there was no increase in HMG-CoA reductase mRNA levels with mitogenic stimulation when cells were cultured in medium supplemented with human lipoproteins. Bovine lipoproteins are able to provide cholesterol to mitogen-stimulated T lymphocytes (J. A. Cuthbert and P. E. Lipsky, unpublished data). Therefore, the finding of an increase in HMG-CoA reductase mRNA with mitogenic stimulation, when cells were cultured in medium supplemented with bovine lipoproteins, is inconsistent with the current results. Regardless of the explanation of the discordant result, the findings in the present experiments clearly show that there is no increase in HMG-CoA reductase mRNA levels when cells are cultured with saturating concentrations of LDL.

The lack of a significant increase in HMG-CoA reductase mRNA levels after mitogenic stimulation in lipoprotein-depleted medium or when LDL cholesterol was present, indicate that HMG-CoA reductase mRNA levels were controlled differently than LDL receptor or HMG-CoA synthase mRNA levels (5). Moreover, the minimal increase in HMG-CoA reductase mRNA levels with mitogenic activation contrasts with the apparent marked increase in functional activity of the sterol biosynthetic pathway and the activity of the rate-limiting enzyme, HMG-CoA reductase [(7), J. A. Cuthbert and P. E. Lipsky, unpublished findings]. Cholesterol biosynthesis increases 20-fold by 24 h after mitogenic stimulation of PBMC (16), the time point chosen for analysis of mRNA. This increase is confined to the responding T cell population (16), is first detected 2–4 h after the addition of mitogen (20), and continues to increase for at least 48 h in culture, at which time the augmentation induced by mitogenic stimulation of T cells is up to 40-fold (16). Increases in mevalonate synthesis, quantitated by measuring HMG-CoA reductase activity, apparently account for almost all the increase in endogenous sterol synthesis with mitogenic stimulation. Thus, in preliminary experiments, HMG-CoA reductase activity was measured in PBMC after a 24-h incubation in lipoprotein-depleted medium. The rate of mevalonate synthesis from radiolabeled HMG-CoA, which reflects activity of HMG-CoA reductase, was approximately 8-fold higher in PHA-stimulated PBMC than in unstimulated PBMC (J. A. Cuthbert and P. E. Lipsky, unpublished data). In addition, whereas mevalonate synthesis is increased by 8-fold, the conversion of mevalonate to sterols is augmented less than 2-fold by mitogenic stimulation (21). Thus, the combination of increased HMG-CoA reductase activity and enhanced synthesis of cholesterol from mevalonate can account for the previously demonstrated 20-fold increase in endogenous sterol synthesis with mitogenic activation of PBMC.

Increases in HMG-CoA reductase mRNA levels do not appear to play an important role in the overall increase in cholesterol biosynthesis in mitogen-activated T lymphocytes. Translational or post-translational increases in HMG-CoA reductase activity are therefore likely to be important in the overall increase in sterol biosynthesis resulting from mitogenic stimulation. In contrast, the thyroid-responsive cell line FRTL-5 manifested an 8-fold increase in sterol synthesis after stimulation by thyrotropin that was associated with an identical 8-fold increase in the rate of HMG-CoA reductase gene transcription (22). Thus, increased sterol biosynthesis after cellular stimulation can apparently be achieved by different mechanisms in different cell populations.

Cellular HMG-CoA reductase activity, and thus sterol biosynthetic rates, can be regulated by at least three different mechanisms (4, 23, 24). In cultured hamster cells, for example, transcription of the gene can be varied by 8-fold (23), translation of the mRNA by 5-fold (23, 24), and degradation of the protein can also be varied by 5-fold (4). In humans, gene transcription produces various sizes of HMG-CoA reductase mRNAs (25–27); however, all sizes of mRNA are coordinately regulated by alterations in sterol metabolism (27). In the current experiments, every HMG-CoA reductase mRNA species was measured by the nuclease protection assay since the single-stranded cDNA probe was synthesized from the middle of the coding region (8, 14). Basal expression of HMG-CoA reductase is apparently governed by transcription factors that interact with sequences in the 5'-flanking region of the gene (28–30). Differences in the level or functional activity of these nuclear transcription factors may eventually explain the observed variability in gene expression after cellular activation in different cell populations.

Sterol-mediated down-regulation of HMG-CoA reductase activity and related sterol synthesis in cultured cells, such as Chinese hamster ovary cells, is achieved not only by decreasing gene transcription but also by enhancing degradation of the enzyme (23–25). In T lymphocytes, LDL cholesterol down-regulates the rate of endogenous sterol synthesis in both unstimulated and mitogen-stimulated T cells to a similar degree (7). Consequently, there remains a 20- to 40-fold increase in endogenous sterol synthesis in mitogen-stimulated cells when compared with resting cells, despite the presence of LDL cholesterol (7). However, mitogen-induced increases in HMG-CoA reductase and synthase mRNA levels were negated by the addition of LDL cholesterol. Therefore, transcriptional changes are unlikely to account for the continued enhancement of sterol synthetic rates with mitogenic activation when exogenous cholesterol was present. Rather, these findings indicate that post-transcriptional mechanisms, such as increases in translation or decreases in degradation of HMG-CoA reductase, are likely to explain the increase in sterol biosynthesis in mitogen-stimulated T lymphocytes.

HMG-CoA synthase activity, unlike HMG-CoA reductase activity, appears to be regulated in vitro almost exclusively by changes in gene transcription (13, 25). In the current experiments, HMG-CoA reductase and synthase levels were coordinately regulated although the extent of the responses was different. Thus, HMG-CoA synthase mRNA levels increased with mitogenic stimulation to a greater degree than HMG-CoA reductase mRNA levels. Consequently, an increase in HMG-CoA synthase activity after mitogenic stimulation may contribute to the observed increase in acetate incorporation into sterols in T lymphocytes cultured in lipoprotein-depleted medium. However, HMG-CoA synthase mRNA levels did not increase with mitogenic stimulation of T cells cultured in medium supplemented with LDL cholesterol. Therefore, a change in HMG-CoA synthase activity is unlikely to contribute to the overall increase in sterol synthesis with mitogenic stimulation when LDL cholesterol is present. Regardless of the contribution of HMG-CoA synthase, the current experiments indicate that any increase in HMG-CoA reductase activity is largely post-transcriptional in origin. The molecular mechanism of post-transcriptional regulation of HMG-CoA reductase appears to be genetically distinct from sterol repression of HMG-CoA reductase gene expression (31). It is possible, therefore, that the genes involved in post-transcriptional regulation are mitogen-responsive whereas those responsible for transcriptional regulation may not be influenced by mitogenic stimulation except in situations where exogenous sterols are limiting.

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