Phospholipid Biosynthesis Program Underlying Membrane Expansion during B-lymphocyte Differentiation*

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Stimulated B-lymphocytes differentiate into plasma cells committed to antibody production. Expansion of the endoplasmic reticulum and Golgi compartments is a prerequisite for high rate synthesis, assembly, and secretion of immunoglobulins. The bacterial cell wall component lipopolysaccharide (LPS) stimulates murine B-cells to proliferate and differentiate into antibody-secreting cells that morphologically resemble plasma cells. LPS activation of CH12 B-cells augmented phospholipid production and initiated a genetic program, including elevated expression of the genes for the synthesis, elongation, and desaturation of fatty acids that supply the phospholipid acyl moieties. Likewise, many of the genes in phospholipid biosynthesis were up-regulated, most notably those encoding Lipin1 and choline phosphotransferase. In contrast, CTP:phosphocholine cytidylyltransferase α (CCTα) protein, a key control point in phosphatidylcholine biosynthesis, increased because of stabilization of protein turnover rather than transcriptional activation. Furthermore, an elevation in cellular diacylglycerol and fatty acid correlated with enhanced allosteric activation of CCTα by the membrane lipids. This work defines a genetic and biochemical program for membrane phospholipid biogenesis that correlates with an increase in the phospholipid components of the endoplasmic reticulum and Golgi compartments in LPS-stimulated B-cells.

The differentiation of a B-lymphocyte into a plasma cell is characterized by a number of events, including expansion of the intracellular membrane network, particularly the rough endoplasmic reticulum (ER),9 where immunoglobulins are synthesized and assembled into functional antibodies. During amplification of the ER, a few resident proteins are expressed preferentially, although the majority increase proportionally to the increased membrane surface area, maintaining the overall membrane protein composition (1). Expression of select targets of the unfolded protein response pathway, a complex interorganelle signaling system that emanates from the ER (2), is triggered during plasma cell differentiation (3). These targets include ER chaperones like BiP and GRP94 and the transcription factor XBP-1 (1, 4–6). XBP-1 is essential for the development of plasma cells (5), pancreatic acinar cells, and salivary gland cells (7), all of which are specialized for secretion and contain an elaborate ER network. The unfolded protein response mediates a novel splicing of XBP-1 mRNA to yield XBP-1(S), a basic leucine zipper protein with a transactivation domain. Indeed, heterologous enforced expression of XBP-1(S) in fibroblasts is sufficient to stimulate synthesis of phosphatidylcholine (PtdCho), the primary phospholipid of the ER membrane, leading to increased intracellular membrane surface area and volume of ER and increased cell size (8, 9). The targets of transcriptional activation that drive membrane phospholipid synthesis during plasma cell maturation remain elusive, however, as expression of the genes of the PtdCho biosynthetic pathway are not significantly stimulated during XBP-1(S) induction (8, 9).

The major route for PtdCho production is the CDP-choline pathway (11), and the supply of CDP-choline is governed by the activity of CCT. Three CCT isoforms are expressed differentially in tissues, but CCTα is the dominant isoform that is expressed ubiquitously (12, 13). Several mechanisms that regulate PtdCho synthesis have been uncovered, and most of these center on CCT. The rate of membrane PtdCho synthesis increases via stabilization of the transcript encoding CCTα when macrophages proliferate in response to colony-stimulating factor-1 (14). Similarly, enhanced expression of CCTα accompanies the stimulation of PtdCho synthesis during neuronal differentiation (13, 15). Stimulation of PtdCho synthesis is associated with cell cycle progression in other cell types (16, 17), and in IIC9 cells it is accompanied by relocation of the CCTα protein from the nucleus to the cytoplasm (18). Reduction of PtdCho synthesis in stimulated pancreatic acini (19), in lung epithelial cells responding to tumor necrosis factor-α (20, 21), or in cells undergoing farnesol-induced apoptosis (22) is

DTT, dithiothreitol; PBS, phosphate-buffered saline; qRT-PCR, quantitative reverse transcription-PCR; IL, interleukin; M, microsomes; C, cytosol; M/C, microsomes to cytosol ratio.
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associated with CCTα reduced activity because of protein degradation. Oxysterols stimulate CCTα phosphorylation, thereby decreasing PtdCho synthesis (23). In addition to mechanisms that regulate CCT protein expression or modification, the biochemical activity of the enzyme is controlled by the membrane lipid composition as CCT responds to both activators and inhibitors embedded in the PtdCho matrix (24, 25). The degree of interaction between membrane lipids and the CCT protein determines the rate of PtdCho synthesis (26) and provides a mechanism for adaptation to changes in the membrane environment. Finally, in the last step of PtdCho synthesis, the CPT uses both CDP-choline and DAG as substrates. Thus, in addition to the supply of CDP-choline, the supply of DAG can be a limiting factor in membrane PtdCho biosynthesis (27–30).

The CH12 cell line, a member of the CH series of murine B-lymphoma cell lines (31), was employed as a model system to look into the changes in lipid metabolism responsible for the acceleration of membrane phospholipid synthesis to support the differentiation of activated B-cells into antibody-secreting plasma cells. Similar to splenic B-cells, CH12 cells bear surface IgM and class II molecules of the major histocompatibility complex, and less than 3% secrete IgM under normal culture conditions (32, 33). Differentiation and Ig secretion are induced either by lipopolysaccharide (LPS) exposure or by T-cells (33–36), and the morphological differentiation is similar to LPS-stimulated splenic B-cells (1). Analysis of the ultrastructural changes of CH12 cells during differentiation showed a 3–6-fold increase in the surface area of ER and Golgi and a similar increase in ER resident proteins as shown by EM and immunoblotting, respectively (1). Our work defines a complex pattern of genetic and biochemical alterations in lipid metabolism that lead to expansion of the intracellular membrane network in LPS-induced differentiation of CH12 B-cells.

EXPERIMENTAL PROCEDURES

CH12 B-cell Culture—The CH12 B-cell lymphoma cell line (31) was maintained by weekly passage as an ascites tumor in B10.A mice (The Jackson Laboratory, Bar Harbor, ME). Cells were harvested by peritoneal lavage and cultured in RPMI 1640 supplemented as described previously (4). CH12 B-cells were seeded at 2 × 10⁶ cells/ml, and differentiation was induced by exposure to 25 μg/ml LPS (Escherichia coli 055:B5, Sigma) (31). Splenic B-cells were isolated from C57BL/6 mice by a depletion strategy using a B-cell isolation kit (Miltenyi Biotech) according to the manufacturer’s protocol. All procedures involving mice were performed according to protocols approved by the Institutional Animal Care and Use Committee of both St. Jude Children’s Research Hospital and Loyola University Medical Center.

Lipid Extraction—CH12 cell pellets (2 × 10⁷ cells) were resuspended in 1 ml of water or PBS. The total volume was measured, and a 0.1-ml aliquot was removed for protein determination. Lipids were extracted from a 900-μl aliquot by the method of Bligh and Dyer (37) using 2.4 ml of acetic acid in methanol (2%, v/v) and 1 ml of chloroform in the first step, followed by 1.5 ml of chloroform and 1.2 ml of water in the second step to yield two phases, organic and aqueous. The organic phase was collected and dried.

Phospholipid Mass and Fatty Acid Distribution—Lipids extracted from CH12 cells were resuspended in 100 μl of chloroform/methanol (2:1, v/v). A 1-μl aliquot was loaded onto a thin layer silica gel rod and developed first in ether, dried, and then developed in chloroform/methanol/acetic acid/water (50: 25:8:2, v/v). Lipids were detected by flame ionization using an Iatroscan Instrument (Iatron Laboratories), and peaks were integrated with PEAKSIMPLE software (SRI Instruments). Peaks were identified by comigration with authentic standards. PtdCho and PtdEtn masses were calculated using standard curves for each.

Lipids extracted from CH12 cells were resuspended in anhydrous methanol and converted into fatty acid methyl esters by addition of few drops of acetyl chloride followed by overnight incubation at room temperature. The solvent was dried under nitrogen, and the methyl esters were recovered by extraction using hexane/water (1:2, v/v). The organic phase was dried under nitrogen, and the methyl esters were dissolved into carbon disulfide and analyzed using a HP 5890 gas chromatograph equipped with a flame ionization detector and a capillary GC column: DB-225, 30 m × 0.53 mm, 0.5 μm (J & W Scientific). Methyl esters were identified by their retention times as determined using gas-liquid chromatography methyl ester standards in the FIM-FAME-7 mixture (Matreya).

Phospholipid Electrospray-MS/MS Analysis—Mass spectrometry (MS) of PtdCho was performed by the Hartwell Center for Bioinformatics and Biotechnology at St. Jude Children’s Research Hospital. Approximately 50 μg of total lipid was dissolved in 0.25 ml of chloroform/methanol 50:50 (v/v) + 1% formic acid. MS analysis was performed using a Finnigan™ TSQ® Quantum (Thermo Electron, San Jose, CA) triple quadrupole mass spectrometer equipped with the nanospray ion source. Samples were introduced via static nanoelectrospray using EconTips™ (New Objective, Woburn, MA). The instrument was operated in the positive ion mode using parent ion scanning for PtdCho. Ion source parameters were as follows: spray voltage 1000 V, capillary temperature 270 °C, capillary offset 35 V, and tube lens offset was set by infusion of the polytyrosine tuning and calibration solution (Thermo Electron, San Jose, CA) in electrospray mode. MS acquisition parameters for PtdCho were as follows: scan range 600–900 m/z, scan time 0.3 s, product mass 184.1 m/z, collision energy 40 V, peak width Q1 and Q3 0.7 FWHM, and Q2 CID gas 0.5 millitorr. Instrument control and data acquisition were performed with the Finnigan™ Xcalibur™ (version 1.4 SR1) software (Thermo Electron, San Jose, CA).

IgM Secretion Rates and Enzyme-linked Immunosorbent Assays—CH12 cells, cultured for 24 and 48 h with or without LPS, were harvested and washed. Cells were subcultured at 2 × 10⁸ viable cells/ml and incubated at 37 °C for 2 h; the amount of IgM secreted in the medium was measured by enzyme-linked immunosorbent assay using goat anti-mouse IgM, μ chain-specific, goat anti-mouse κ-alkaline phosphatase, κ chain-specific (SouthernBiotech, Birmingham, AL), and 4-methylumbelliferyl phosphate (Sigma) as substrate.

Enzyme Assays—CK and CCT activities in CH12 cell lysates were measured as described previously (8). The activation of CCT by CH12 cell lipids was measured using purified recom-
binant CCTα protein. Recombinant rodent CCTα was expressed as histidine-tagged protein in SF9 insect cells and purified by metal-affinity and size-exclusion chromatographies using a HiTrap™ chelating HP column and a HiLoad™ 16/60 Superdex™ 200 column (Amersham Biosciences). Briefly, enzymatic activity was measured by incubation at 37 °C for 30 min of 0.5 μg/ml recombinant CCTα in assay buffer (100 mM HEPES, pH 7.4, 100 mM KCl, 20 mM MgCl2, 1 mM EGTA) in the presence of 1 mM CTP, 1 mM phosphocholine labeled with 3.2 μCi/mmol [32P]phosphatidic acid product, and 1% HClO4 (1:1, v/v). After centrifugation at 5,000 rpm for 30 min, the reaction was stopped by the addition of chloroform/methanol (1:2, v/v) and 1% HClO4, and the final phospholipid concentration of 1 mg/ml by vortexing and sonication. LUVs were extruded through a 100 nm pore diameter polycarbonate membrane, and the phospholipid concentration was determined colorimetrically using ammonium ferrirothiocyanate (38). LUVs were stored at 4 °C and used within 2 days of preparation. CPT activity in CH12 microsomes, prepared from frozen cell pellets, was determined as described previously (8, 39).

DAG Quantification—DAG was quantified in lipids extracted from cells according to the protocol of Preiss et al. (40). The extracted samples or 1,2-dioleoyl-sn-glycerol standard (Avanti, Alabaster, AL) were solubilized in octyl-β-D-glucoside/cardiolipin, and DAG mass was determined by enzymatic assay in a reaction buffer containing 100 mM imidazole HCl, 100 mM NaCl, 25 mM MgCl2, 2 mM EGTA, pH 6.6, 20 mM DTT, DAG kinase (Calbiochem), and 10 mM ATP plus 1 μCi of [γ-32P]ATP (30 Ci/ml). The reaction was incubated at room temperature for 30 min. The reaction was stopped by the addition of chloroform/methanol (1:2, v/v) and 1% HClO4, and the [32P]phosphatidic acid product was extracted using chloroform, 1% HClO4 (1:1, v/v). After centrifugation at 5,000 × g to separate the phases, the upper layer was discarded, and the lower layer, which contained [32P]phosphatidic acid product, was washed twice with 1% HClO4 and dried under vacuum. The dried samples were dissolved in 5% methanol in chloroform and fractionated on TLC plates developed in chloroform/methanol/acetic acid (65:27:8, v/v). Spots corresponding to the phosphatidic acid were scraped and counted for the radioactivity using scintillation spectrometry.

Metabolic Labeling—CH12 B-cells were seeded at 2 × 10^5 cells/ml in medium with or without 25 μg/ml LPS. At times after LPS addition, cells were harvested and resuspended at 10^5 cells/ml in 50 μl of labeling medium as follows: choline-free culture medium supplemented with 2 mM EGTA, 2 mM DTT, 80 μM digitonin, 0.02% Tween 20, and 100 μM [14C]CDP-choline 0.9 μCi/ml (American Radiolabeled Chemicals). Cells were incubated for 4 min at 37 °C; reactions were stopped by adding 240 μl of 2% acetic acid in methanol. Lipids were extracted as described earlier, and the amount of choline incorporated into PtdCho was estimated by scintillation spectrometry.

RNA Transcript Measurements—Total RNA was isolated from CH12 B-cells cultured with or without LPS for 3, 24, or 48 h using TRIzol (Invitrogen); contaminating genomic DNA was removed by digestion with DNase I, and aliquots were stored as an ethanol precipitate at −20 °C. cDNA was prepared from RNA by reverse transcription using SuperScript II RNase H− reverse transcriptase (Invitrogen) and random primers. Primers and probes for real time qRT-PCR were designed using Primer Express® software (version 2.0; Applied Biosystems) and are listed in Table 1. Real time qRT-PCR was carried out using the 7300 Real Time PCR System and 7300 System SDS software (version 1.2.3; Applied Biosystems). The Taqman Rodent GAPDH control reagent (Applied Biosystems) was the source of the primers and probes for quantifying the control Gapdh mRNA. The collected data were analyzed using the C_T method (41); the amount of target RNA was normalized to the endogenous Gapdh reference and related to the amount of target RNA in untreated cells. The specific number of experiments (n) and p values for statistical significance as evaluated by Student’s t test (unpaired) are reported in each legend; the following convention was used for representing significance: * indicates 0.01 < p < 0.05; ** indicates 0.001 < p < 0.01, and *** indicates p < 0.001.
**Affymetrix Array Analysis**—Following the manufacturer’s protocol, total RNA was used to prepare cRNA for hybridization, washing, and scanning of a GeneChip® Mouse Genome 430 2.0 array (Affymetrix, Inc., Santa Clara, CA) using a GeneChip® Fluidics Station 400 and a GeneArray™ scanner. Data were collected using Microarray Suite software (formerly known as GeneChip® Suite software). Comparison and statistical analysis of all the Affymetrix data were achieved using Spotfire® DecisionSite® 8.11 (Spotfire, Inc.) software. The specific number of experiments (n) and p values for statistical significance as evaluated by Student’s t test (unpaired) are reported in the footnotes to Table 2.

**Immunoprecipitation**—CH12 cells were cultured with LPS for 0, 24, and 48 h. At each time point, cycloheximide (CHX) was added to the medium to a final concentration of 100 μg/ml, and the cells were harvested after 1–3 h of incubation. Pellets containing the same cell number (2 × 10^7) were then lysed with 1.4 ml of ice-cold lysis buffer (50 mM Tris-HCl, pH 8.0, 5 mM EDTA, 0.1 mM Na_2VO_4, 50 mM NaF, Complete Inhibitor Mixture (Roche Applied Science), 5 mM DTT, 1 mM phenylmethylsulfonyl fluoride, and 0.1% Triton X-100). CCTα were then separated by 8% SDS-PAGE and transferred onto Immuno-Blot PVDF membrane (Bio-Rad). The membrane was blocked with a suspension of 5% dry milk in 20 mM Tris-HCl, pH 7.4, 137 mM NaCl, and 0.1% (v/v) Tween 20 (TBS-T) and washed with TBS-T. The membrane was then incubated for 1.5 h with anti-CCTα antibody (5 μg/ml in TBS-T+1% dry milk), washed with TBS-T, incubated for 1 h with protein A-horseradish peroxidase (Sigma, 1 μg/ml in TBS-T+1% dry milk), and washed with TBS-T. Protein A was detected by chemiluminescence using the ECL Plus Western blotting detection system (Amersham Biosciences) and Kodak Biomax MR film. CCTα band intensities were analyzed by ImageQuant software, version 5.2 (Amersham Biosciences), and the protein half-life was estimated by one-phase exponential decay analysis using Prism 4 software, version 4.00 (GraphPad software, Inc.). Cell lysates were prepared as described previously (42). Chemiluminescent immunoblotting of Ig μ and κ chains, GRP94, BiP, TRAPα, XBP-1, and β-actin was performed as described (42).

**Immunohistochemistry**—CH12 cells were exposed to LPS for 0 and 48 h, harvested, and fixed to glass slides using a cytopsin at 500 × g for 5 min. Cells were fixed with methanol/acetic acid (3:1, v/v) two times for 10 min and then washed with water three times for 10 min and air-dried. The slides were incubated with 1 mg/ml NaBH_4 in PBS two times for 5 min and then...
washed three times for 5 min with PBS to reduce autofluorescence. CCTα was localized by incubation with rabbit anti-CCTα followed by goat anti-rabbit Ig linked to AlexaFluor 564 (Molecular Probes). IgM was localized by incubation with goat anti-mouse IgM AlexaFluor 488 (Molecular Probes). Slides were mounted with ProLong® Gold antifade reagent with or without DAPI (Molecular Probes). Confocal microscopy was performed by the Scientific Imaging Shared Resource at St. Jude Children’s Research Hospital using a Zeiss LSM 510 META multiphoton microscope equipped with a Plan-Neofluor ×40/1.3 oil objective and controlled by laser scanning microscope LSM 510 software (version 3.2 SP@) (Carl Zeiss GmbH, Germany).

**Electron Microscopy**—After 48 h of culture in medium alone or in the presence of LPS or IL-5, CH12 cells were harvested and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer. After fixation, the cells were spun down, placed in 2% agar, and rinsed in three 10-min changes of 0.1 M sodium cacodylate buffer with 5% sucrose. Cells were post-fixed in 1% osmium tetroxide in the same buffer for 1 h and then rinsed twice for 10 min in buffer. After running through a graded series of alcohol and clearing in propylene oxide, samples were infiltrated with equal parts Epon araldite/propylene oxide, followed by two changes of 100% Epon araldite. The samples were embedded in fresh plastic and polymerized at 80 °C overnight. Sample sections, 70 nm thickness, were analyzed using a Jeol 1200EX 125KV electron microscope equipped with an AMTV542 2K camera (Advanced Microscopy Techniques Corp.) at the Integrated Microscopy Center at the University of Memphis, Tennessee.

**RESULTS**

**Lipid Biogenesis**—CH12 B-cells were cultured with or without LPS for 24 and 48 h. Membrane lipid composition was then measured following solvent extraction and chromatography analysis. PtdCho (55% of the total lipid) was the major membrane lipid in CH12 B-cells, and the cellular content of PtdCho increased significantly following LPS treatment (Fig. 1A). PtdEtn, the second major component (15% of the total lipid), followed suit. Cholesterol was 2.5% of total lipids and also increased. Cholesterol esters did not change, and triglycerides, diacylglycerols, free fatty acids, and other lipid species were not reliably quantified using this methodology. Data in the inset of Fig. 1A illustrate that there was not an increase in the phospholipid or cholesterol components when CH12 B-cells were cultured without LPS. The cells accumulated proteins as well as lipids after 24 and 48 h of LPS stimulation (Fig. 1C), and the proteins represented both resident ER proteins and newly synthesized Ig proteins destined for secretion. The kinetics of protein accumulation did not strictly parallel the kinetics of lipid accumulation (Fig. 1, A and C). Newly synthesized IgM was secreted at a rate of 13 μg/h/10⁷ cells at 24 h after LPS, and the rate doubled at 48 h after LPS (Fig. 1D), correlating with the lower protein content per cell at 48 h (Fig. 1C). PtdCho and PtdEtn were significantly more than the cellular protein content at 48 h following LPS (Fig. 1B), which correlated with expansion of the intracellular membrane network (see Fig. 9 (43)). A change in the cellular lipid content was not observed in CH12 cells cultured for the same length of time in the absence of LPS (Fig. 1, A and B, insets). These data establish that the formation of phospholipid is significantly activated to provide the bulk membrane lipid components required for the expansion of the ER and Golgi compartment in LPS-stimulated CH12 B-cells.

**Gene Expression Analysis**—Affymetrix arrays were used to first survey the expression of lipogenic genes. CH12 B-cells cultured for 48 h with LPS were compared with control cells cultured without LPS. A global view of the gene expression changes using gene ontology analysis (44) showed, as expected, that the most significant biological process affected by LPS stimulation was the defensive immune response to biotic stimuli (p < 4.6 × 10⁻²⁰), and the most significant cellular components affected were the endoplasmic reticulum (p < 1 × 10⁻¹²) and the nuclear envelope-endoplasmic reticulum network (p < 2 × 10⁻¹⁹). We focused on the genes involved in bulk lipid production and used the array data to identify the isoforms of these enzymes expressed in the CH12 cells. This subset of genes that was significantly affected by LPS exposure for 24 and 48 h is shown in Table 2. These data revealed a pattern indicating up-regulation of specific isozymes in FA synthesis, FA modification, and the CDP-choline pathway of PtdCho biosynthesis. The transcripts that reliably changed by about 2-fold or more among replicate experiments are listed. Notably, expression of genes encoding enzymes in the CDP-ethanolamine pathway or the serine decarboxylase pathway of PtdEtn biosynthesis did not change significantly, nor did the PtdEttn methyltransferase transcripts. We next investigated the individual genes encoding each of the key pathway enzymes using real time qRT-PCR to quantify the changes in transcript levels over time, and we correlated these changes with biochemical rate measurements where possible. We also investigated expression of genes that were of potential importance in lipid synthesis but did not signal as changed on the microarray to confirm the data.

**Regulation of PtdCho Synthesis**—PtdCho is synthesized through two pathways, the CDP-choline pathway and the PtdEtn methylation pathway. The main route to PtdCho in B-cells is the CDP-choline pathway (45), which synthesizes PtdCho via CK, CCT, and CPT. Cultured CH12 cells were dependent on this pathway because they did not survive in *in vitro* culture if the medium was choline-deficient (data not shown). PtdCho synthesis via the CDP-choline pathway, as well as the distribution of PtdCho precursors, was determined by labeling the cells for 2-, 2.5-, and 3.0-h intervals with 6 μM [³H]choline at various time points after the addition of LPS. After 3 h of incubation, CDP-choline was the smallest component (Fig. 2A), and its content was not different between treated and untreated cells (Fig. 2B, gray bars). However, the relative rate of CDP-choline synthesis was greater in LPS-treated cells (cells treated with LPS divided by cells without LPS) and increased 2.5-fold by 48 h after LPS treatment (Fig. 2B, white bars). The rate of radiolabeled choline incorporation into PtdCho increased by almost 2- and 6-fold at 24 and 48 h, respectively, compared with the rate at time 0 (Fig. 2B). These data indicated that the increased activity of the CDP-choline pathway correlated with the net increase in PtdCho following the LPS stimulation (Fig. 1). The relatively large amount of radiolabeled P-choline compared with the small amount of CDP-choline demonstrated not only active CK but that the constrictive point, or rate-limiting step, in the pathway was CCT (Fig. 2A). The small size of the
radiolabeled CDP-choline pool indicated that the product of the CCT enzyme was rapidly converted into PtdCho. It is the slow step that determines the rate of flux through any pathway, and these data indicated that the CCT activity had to change to produce more PtdCho. To confirm that CDP-choline synthesis was the rate-limiting step in vivo, permeabilized CH12 cells were incubated for 4 min with an excess supply of [14C]CDP-choline to measure CPT/CEPT activity. The rate of choline incorporation into PtdCho in stimulated, permeabilized cells (Fig. 2D) was 100 times higher than in intact cells (Fig. 2C) and illustrated that the supply of CDP-choline in intact cells governed the rate of PtdCho synthesis, despite an increased abundance of CPT. Addition of exogenous DAG to the assay did not change the rate of PtdCho synthesis, indicating that the amount of endogenous DAG was sufficient during these short term measurements. Cells labeled with [3H]ethanolamine at times during a 48-h LPS exposure showed that the rate of PtdEtn conversion into PtdCho, in pmol/h/10^7 cells, was as follows: 2 (6% of the PtdEtn) at 0 h, 8 (9%) at 6 h, 58 (7%) at 24 h, and 101 (10%) at 48 h. Thus, there was increased conversion that was mediated by PtdEtn methyltransferase, but it contributed only 2–3% of the total cellular PtdCho, and this route of synthesis alone could not account for the dramatic increase in PtdCho.

Expression of Enzymes for PtdCho Synthesis—The catalytic activities of the CDP-choline pathway were determined in lysates from LPS-treated cells as a measure of protein expression. CPT, CK, and CCT enzyme-specific activities were measured in vitro (Fig. 2E). Cellular CPT activity increased 3-fold at 24 h and 6-fold at 48 h after LPS exposure (Fig. 2E). CPT is an integral membrane protein (46), and the increased CPT activity at 48 h correlated with expansion of the ER and Golgi (43). CK
induced differentiation, with CPT exhibiting the highest rise. The increased CK and CPT capacities worked in conjunction with the rate-limiting CCT to augment PtdCho synthesis.

The contribution of gene expression to the activity of the CDP-choline pathway was assessed using qRT-PCR to quantify mRNA levels following LPS stimulation. The genes encoding both the CKα (Chka) and CKβ (Chkb) isoforms were expressed in the absence of LPS, and a transient 2-fold increase in Chka gene transcripts was observed, peaking at 3 h after LPS exposure (Fig. 3A). The increase in CKα isoform expression preceded the increased CK activity (Fig. 2E). The Pcyt1b gene encoding the CCTβ isoform was not expressed in the CH12 cells, but the Pcyt1a gene encoding the CCTα was expressed, and it did not change significantly following LPS (data not shown). These data suggested that the increased flux through the CCT step following LPS treatment was not because of an increase in Pcyt1a mRNA and indicated that mechanisms other than gene expression were regulating this rate-limiting enzyme. The expression of the Chpt1 gene encoding the CPT1 isoform increased between 5- and 7-fold between 24 and 48 h of differentiation (Fig. 3B), but the Cept1 gene expression encoding the choline/ethanolamine phosphotransferase isoform was not significantly altered (data not shown). Thus, CPT was the most up-regulated enzyme in the pathway by gene expression. The qRT-PCR measurements confirmed that the genes encoding the enzymes leading to PtdEtn and the PtdEtn methyltransferase did not show any difference in their level of expression as a function of LPS (data not shown).

**Activation of CCT**—The 40% increase in total cellular CCT activity indicated some elevation in CCTα protein, but there was no indication that the Pcyt1a gene was activated following LPS treatment. CCTα protein levels in CH12 B-cells before and after LPS stimulation were determined by immunoblotting. CCTα migration on gels is often characterized by two slower migrating bands that represent different phosphorylation states of the protein (47). Phosphorylation occurs exclusively at multiple sites on the carboxyl terminus (48) and reduces but does not eradicate the enzyme activity (49–51). Immunoblotting at 48 h after LPS (Fig. 4) revealed a greater degree of phosphorylation compared with CH12 B-cells at zero time. The half-life of the CCTα protein in the presence of cycloheximide was 1.4 h, but the half-life of CCTα was significantly extended.
within 24 h after LPS (Fig. 4). Furthermore, real time qRT-PCR analysis indicated no change in Pcyt1a expression following cycloheximide exposure (data not shown). These data indicated that the increase in total cellular CCTα activity was because of stabilization of protein turnover, rather than an increase in protein half-lives were calculated from these data as described under “Experimental Procedures.” The average of the three independent experiments ± S.D. is shown below the immunoblot for cells without LPS treatment or 24 or 48 h after LPS. CCTα degradation was not observed in any of the 24- and 48-h samples, and >16 h is used to indicate that the half-life is longer than the average cell doubling time.

**FIGURE 5.** Stabilization of CCTα protein during LPS-induced differentiation. CH12 B-cells were exposed to LPS for 24 and 48 h, and at each time point CHX was added to the medium, and the cells were harvested after 0, 1, 2, and 3 h. Cells were lysed and CCTα recovered by immunoprecipitation of equal numbers of cells using a previously characterized antibody raised against full-length recombinant rodent CCTα (55). Immunoprecipitated CCTα protein was fractionated by SDS-PAGE and immunoblotted as described under “Experimental Procedures.” The three bands representing CCTα (arrows) were quantified and normalized to the initial protein level without CHX at each 0-h time point (bar graph). The illustrated immunoblot and the corresponding bar graph represent one of three independent experiments. The protein half-lives were calculated from these data as described under “Experimental Procedures.” The average of the three independent experiments ± S.D. is shown below the immunoblot for cells without LPS treatment or 24 or 48 h after LPS. CCTα degradation was not observed in any of the 24- and 48-h samples, and >16 h is used to indicate that the half-life is longer than the average cell doubling time.

**FIGURE 4.** Intracellular location of CCTα in CH12 and primary splenic B-cells. CCTα (green) and IgM (red) were localized by immunostaining untreated CH12 B-cells (row A) and cells exposed to LPS for 48 h (row B). Untreated primary splenic B-cells are shown in row C. The nuclei of splenic B-cells were stained with DAPI (C3 and C4, blue), and the contours of the CH12 cells were defined by bright field images (A4 and B4). CCTα, IgM, and DAPI/bright field images were merged and are shown in A3 and B3. The experiment was repeated twice with two different fixation methods with the same results.

CCTα protein is found either in the cytoplasm, in association with organelle membranes, or in the nuclear matrix. In many cell types, nuclear CCTα dominates (52, 53) and is transported to this site via a nuclear localization signal at its amino terminus (54). Lung epithelia are an exception where CCTα is located exclusively outside the nucleus where a high rate of PtdCho formation is occurring to support the secretion of lung surfactant (55). Differentiating B-cells also have high rates of PtdCho synthesis (56) (Fig. 2A), and we found CCTα to be located outside the nucleus in CH12 B-cells in proximity to the expanding extranuclear membrane compartment. An antibody specific for the CCTα catalytic region was used for in situ immunofluorescent localization, and the protein was predominantly found outside the nucleus both before (Fig. 5A1) and after LPS treatment (Fig. 5B1). The protein co-localized with the IgM synthesized in the ER both before and during differentiation (Fig. 5, A3 and B3), and these data showed that CCTα protein was in proximal association with the ER membranes in CH12 cells rather than being translocated to the nucleus. Likewise, analysis of primary naive murine splenic B-cells revealed that CCTα was also located outside of the nucleus and associated with the IgM counterstain (Fig. 5C3).

CCTα can associate with membranes, and the membrane lipid composition is a major determinant of its enzyme activity (24). We were unable to evaluate the CCT activity following LPS in digitonin-permeabilized cells, and so we fractionated B-cell lysates into membrane and cytoplasmic components to measure the total CCT activity associated with each (Table 3). The proportion of membrane activity (M:C) was higher in LPS-treated samples at 24 h, and at 48 h the LPS-treated values matched those of untreated fractionated lysates. These determinations reflected the effects of endogenous lipid, because no additional lipid vesicles were added to the assays, and the lipids associated with the different fractions were not stripped away by detergent solubilization. The addition of the PtdCho/oleic acid maximum activator revealed the total activity associated with each fraction, and membrane-associated CCT was slightly higher in lysates prepared from LPS-treated versus untreated cells. The data with lipid activator point out the 40% increase in CCT activity that was observed in total cell lysates following LPS (Fig. 2E) but suggest that the distribution of protein between membrane and cytoplasm was not as biased as the endogenous lipid activation of each fraction.
Purified CCT has low activity when exposed to vesicles made up of PtdCho alone, but it is activated several orders of magnitude by the addition of specific lipids, such as DAG or fatty acid, to the vesicles. Thus, alterations in membrane lipid composition in cells may contribute to the stimulation of activity and flux through the CCT step during B-cell differentiation. We tested whether the lipid composition of B-cell membranes influenced CCTα activity using purified, lipid-depleted recombinant CCTα. Lipids extracted from B-cells at 0, 24, and 48 h after LPS treatment activated CCTα to different extents. The half-maximal stimulatory activity for the lipid preparations, measured as the lipid concentration required to reach half of the maximum enzyme velocity, decreased from 15 μM in untreated cells to 7 μM 48 h after LPS stimulation (Fig. 6A). The amount of lipid vesicles added to each assay was based on the phospholipid content, and thus the data indicated the presence of higher proportions of lipid activators that would promote CCTα membrane association. The bulk composition of the lipid vesicles is illustrated in Fig. 1, but the amphiphiles known to be most potent were below the detection limits of those determinations. Thus, we used the more sensitive DAG kinase assay to measure DAG, one of the known activators presumably in the cellular lipid mixture. The cellular DAG content increased from 2.0 ± 0.2 nmol/10^7 cells at time 0 to 8.8 ± 0.2 nmol/10^7 cells at 48 h (p < 0.0001; n = 8) after LPS treatment (Fig. 6A). These data mirrored the increased affinity of CCTα for the membrane lipids (Fig. 6A). Free FA is another amphiphile that is known to activate CCT, and so radiolabeling was used to measure both DAG and FA synthesis at times following LPS. The incorporation of [1^14]C]acetate into FAs, DAG, and the total

### TABLE 3

**CCT activity distribution in CH12 B-cells cultured ± LPS for 24 or 48 h**

CH12 cells were harvested at 24 or 48 h after culture ± LPS. Liposomes were recovered by centrifugation as described under “Experimental Procedures.” CCT activity in the cytosol (C) and microsomes (M) was measured with or without lipid activator (DOPC/OA). Total activity (C + M) and the microsomes to cytosol ratio (M:C) were calculated.

| Hours in culture | Lipid activator | CCT activity (nmol/h/10^7 cells) | LPS-treated | Untreated |
|-----------------|----------------|---------------------------------|-------------|----------|
|                 |                | Total M:C                       | Total M:C   |           |
| 24              | −              | 10.3 ± 0.2                      | 22.3 ± 0.6  | 7.0 ± 1.1 |
| 48              | −              | 9.0 ± 0.5                       | 7.2 ± 0.4   | 6.9 ± 0.4 |
| 24              | +              | 20.5 ± 0.2                      | 1.55 ± 0.03 | 16.9 ± 1.8|
| 48              | +              | 21.8 ± 1.5                      | 0.79 ± 0.12 | 14.2 ± 0.7 |

**FIGURE 6.** **CCTα regulation by endogenous lipids.** Cells were cultured with LPS for 0, 24, and 48 h, and lipids were extracted, and the amount of phospholipids was quantified as described under “Experimental Procedures.” A, half-maximal stimulatory activity (●) of purified recombinant CCTα in response to LUVs prepared from CH12 cellular lipids at indicated times after LPS treatment. DAG content in the lipid fraction is shown by ○. The data represent the mean of five determinations ± S.D. and are representative of two independent experiments. B, cells were cultured with LPS for 0, 24, and 48 h and pulsed with 2 μCi/ml [2^14]C]acetate for 2 h at each time point. Lipids were extracted, fractionated by TLC, and quantified as described under “Experimental Procedures.” The rates of DAG (●), FA (○), and total lipid (■) synthesis were normalized to 10^7 cells. C and D, total RNA was isolated from cells cultured with or without LPS for 3, 24, and 48 h and real time qRT-PCR with gene-specific primers, and probes were used to quantify the transcript levels relative to Gapdh of PtdOH phosphatases capable of generating DAG and to quantify the change in each gene transcript relative to the level in samples without LPS. C, Ppap2b transcripts encode the PtdOH phosphatase 2b isoform; D, Lpin1 transcripts encode the Lipin1 PtdOH phosphatase. The results are the mean values ± S.E. from three determinations and are representative of at least two independent experiments.
FIGURE 7. Up-regulation of genes involved in FA formation and modification. Total RNA was isolated from cells cultured with or without LPS for 3, 24, and 48 h. Real time qRT-PCR with gene-specific primers and probes was used to quantify the initial transcript levels relative to Gapdh expression and to quantify the change in each gene transcript relative to untreated cells. A, Fasn, FA synthase transcript; B, Elov6, microsomal fatty acid elongase 6 transcript; C, Pecr, peroxisomal enoyl-CoA reductase; D, Fads2, fatty acid desaturase 2 transcript; E, Scd2, stearoyl-CoA desaturase 2 transcript. The results are the mean values ± S.E. from three determinations and representative of at least two independent experiments.

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lipid fraction was evaluated in 2-h pulse-labeling experiments (Fig. 6B). The rate of synthesis of lipid acyl groups increased substantially as early as 6 h after LPS stimulation and remained elevated thereafter. The rate of DAG synthesis increased at 6 h and remained elevated throughout the 48 h of LPS treatment. Free FAs were also able to accumulate at an increased rate at 24 and 48 h after LPS. On the other hand, PtdEtn is also an activator compared with PtdCho, and although it is less potent than DAG and FA, it is more abundant. The data in Fig. 1 indicated that the fraction of PtdEtn relative to PtdCho did not increase after LPS, however. There may have been other amphiphiles in the lipid mixture that as yet are unidentified as activators and that are below the limits of detection. Nevertheless, these would be included in the lipids derived from the stimulated CH12 cells, and altogether, the lipid mixture promotes an increase in CCT enzymatic activity after LPS stimulation.

Production of the Lipogenic Components following LPS Stimulation—DAG in the de novo pathway of phospholipid biosynthesis is produced by PtdOH phosphatase activity, and there are several isoforms expressed in CH12 B-cells. The predominant isozyme was Ppap2b, which was expressed at 30-fold higher levels than Ppap2a, and >100-fold higher than Lpin1. Although the expression of Ppap2a remained constant (data not shown) during the 48-h culture of both treated and untreated cells, Ppap2b and Lpin1 transcripts underwent a striking differential regulation. Compared with untreated cells, Ppap2b transcripts decreased significantly, as denoted by a ratio <1.0 (Fig. 6C). The expression of the Lpin1 gene, recently identified as encoding a PtdOH phosphatase (57), increased by 7-fold following LPS exposure (Fig. 6D). The expression of the Lipin1 phosphatase and the CPT in the CDP-choline pathway displayed the largest overall increases in gene expression and were stimulated in a coordinate manner. These data suggested that Lipin1 was an important phosphatase activity that responded to LPS to stimulate DAG production.

The increase in bulk phospholipid following LPS required increased input of fatty acids as well as phospholipid head groups. The genetic up-regulation of the enzymes responsible for FA synthesis and modification (Fig. 7) contributed to increased de novo synthesis of membrane phospholipid. FA synthase (Fasn) transcripts increased early following LPS treatment compared with controls (Fig. 7A). Temporally downstream of the increase in Fasn expression were significant increases in two genes that elon-
evaluate their responses to LPS. PUFAs accounted for 44% of the total FA in mouse serum, whereas the serum used in cell culture contained only 7% PUFA (data not shown). Thus, both control and LPS-stimulated B-cells exhibited a decrease in PUFA and an increase in MUFA during cell culture, and LPS stimulation resulted in an acceleration of this change. In LPS-stimulated cells, PUFA decreased from 40.6 ± 0.5% at time 0 to 14.1 ± 1.0% at 48 h, and MUFA increased from 18.6 ± 0.2 to 39.3 ± 2.8% in the same time frame. In unstimulated cells, MUFA was 32.1 ± 1.3% of the total, reflecting the same trend but to a lesser extent because of the lower levels of PtdCho synthesis in unstimulated cells. The ratio of saturated/unsaturated fatty acid at time 0 (1.4) and at 48 h (1.1) was modestly altered in both the control and LPS-stimulated cells. These FA compositional changes were clearly reflected in the PtdCho molecular species profile (Fig. 8). PtdCho molecular species containing 18:2, 20:4, and 22:6 fatty acids were abundant in CH12 cells when they were harvested from mice (Fig. 8A). At 24 h, these molecular species had diminished in control cells (Fig. 8B), and this effect was exacerbated by LPS stimulation (Fig. 8C). These data support the idea that increased PtdCho production for ER membrane formation was supported by the acceleration of the de novo FA synthesis and modification systems.

Comparison of LPS- and IL-5-induced B-cell Differentiation—B-lymphocytes can be stimulated by a number of ligands to secrete Ig proteins. For example, CH12 B-cells differentiate into Ig-secreting cells in response to LPS, interleukin (IL)-5, and IL-6 (58). Thus we compared the lipogenic responses of CH12 B-cells stimulated by LPS and by IL-5 to determine whether a selection of those parameters we identified as important for the response to LPS were generally important for plasmablast differentiation. LPS and IL-5 treatment resulted in similar morphological changes as illustrated in Fig. 9. Unstimulated cells (Fig. 9A) were smaller, and the region outside the nucleus, which contained the extranuclear organelles including ER and Golgi, was considerably smaller than the nucleus. Stimulation with either LPS (Fig. 9B) or IL-5 (Fig. 9C) for 48 h resulted in a dramatic increase in cell size, largely because of expansion of the region outside the nucleus. The cytoplasm of the stimulated cells contained a large number of ER membranous structures as well as more mitochondria. Immunoblot analysis of CH12 B-cells was done with resting cells and following culture without treatment, treatment with LPS, or treatment with IL-5 for 48 h (Fig. 10A). These data showed increased expression of the ER-soluble chaperone proteins GRP94 and BiP, a component of the ER-associated translocon TRAPα, secretory IgM heavy chain μκ, the Ig κ light chain κ, and the unfolded protein response-associated splice variant transcription factor XBP-1(S), following stimulation with either ligand. The rates of IgM secretion from both LPS- and IL-5-stimulated cells increased as well as 24 h, and LPS stimulation resulted in a significant increase in cell surface area (Fig. 9B and C). The expression of two key genes, Chpt1 and Lpin1, also increased after 48 h of IL-5 stimulation (Fig. 10D). Transcripts for these genes encoding the CPT enzyme and the Lipin1 phosphatase did not increase relative to untreated controls at 24 h, however, and these kinetics were similar to those following LPS stimulation (Fig. 3 and 6). These data support the concept that many of
the changes outlined in this study underlie B-cell differentiation into plasmablasts that secrete IgM.

**DISCUSSION**

Our analysis of lipid metabolism in CH12 B-cells defines a set of genetic components that lead to the increase in phospholipid synthesis during LPS-induced differentiation (Fig. 11). The switch to high rate antibody synthesis and secretion in the terminal differentiation of B-lymphocytes into antibody-producing plasma cells is accompanied by expansion of the ER and Golgi compartments (Fig. 10) (1). The increase in PtdCho that is destined for the Golgi and ER compartments correlates with transient elevation of CK transcripts leading to a modest 70% increase in protein expression (Fig. 2E) and a progressive and significant increase in CPT transcripts and protein (Fig. 2F). However, a combination of biochemical mechanisms stimulate the CCT (Fig. 2E, Table 3, and Figs. 4 and 6) that determines the overall rate of PtdCho synthesis. CCT protein levels modestly increase by 40% due in part to decreased protein degradation and significant increase in protein half-life following LPS stimulation (Fig. 4). Our data do not address the possibility of increased CCT translation as a contributing factor, as it occurs in XBP-1(S)-transduced fibroblasts (59). The association between cellular phospholipid plus activating lipid amphiphiles and CCT protein is also enhanced (Fig. 6A), thereby increasing activity (Table 3). The combination of these processes contributes to an increased rate of CDP-choline synthesis in intact cells (Fig. 2B). An increase in the association of CCT with lipids has been reported to confer resistance to proteolytic digestion (60); however, the measurement of total protein activity in the membrane and cytoplasmic fractions under optimal conditions (Table 3) would suggest that the resistance to proteolysis is not because of an increased ratio of CCT that is membrane-associated. Rather, LPS induces the stabilization of a number of B-cell proteins by a general mechanism (61), and CCT is likely included in this subset.

The bulk lipid increases after LPS stimulation, but the bulk composition does not change substantially (Fig. 1). Rather, more subtle changes in cellular lipid composition contribute to the clear stimulation of CCT activity (Figs. 2B and 6A). Among these changes, we measured increased accumulation of DAG and free FA (Fig. 6, A and B), two lipids that activate CCT potently when embedded in a PtdCho matrix (25, 26) (Table 3 and Fig. 6, A and B). Replacement of PUFA and longer chain acyl groups with MUFA and shorter chain acyl groups on PtdCho (Fig. 8) may also contribute to the lipid-mediated stimulation of CCT, along with possible trace amounts of other phospholipids such as phosphatidic acid, phosphatidylglycerol, or lipids that as yet are unidentified. Nevertheless, the direct test of the hypothesis in Fig. 6A shows that the cellular lipid mixture contributes to activation of the CCT after LPS stimulation.

So what factors contribute to elevating the DAG and FA levels? Our data indicate that among the PtdOH phosphatases expressed in CH12 cells, expression of the Lipin1 isoform is...
responsive to LPS in a positive manner (Fig. 6, C and D). To the best of our knowledge, DAG is produced in the de novo pathway of phospholipid synthesis by the action of the PtdOH phosphatases (Fig. 11). DAG can also be derived from phospholipase degradation of pre-existing phospholipid, but we focused on the possible targets in de novo synthesis because the Affymetrix analysis did not indicate any changes in phospholipase C or D expression, and because phospholipase action would decrease rather than increase the amount of phospholipid in cells. The DAG has two roles during lipogenesis in the B-cell system. First, it is a required precursor for bulk phospholipid production as a co-substrate for the CPT together with CDP-choline. Second, it is an activator of the CCT step. The FA also has two roles during lipogenesis. First, it is a de novo precursor for PtdOH and DAG (Fig. 11). Second, it is also an activator of the CCT. As intermediates in the phospholipid biosynthetic pathway, DAG and FA could be considered as feedforward regulators, and mechanisms that enhance their accumulation would have a positive effect on PtdCho production.

DAG, as a key intermediate in phospholipid synthesis, has been reported to increase in conjunction with increased PtdCho synthesis (27, 62, 63), and our data are in agreement (Fig. 6, A and B). It is interesting that the DAG elevation is accompanied by down-regulation of Ppap2b expression, the principal

PTdOH phosphatase found in CH12 cells, and an equally pronounced up-regulation of Lipin1 (Fig. 6, C and D). Both of these genes encode PTdOH phosphatases (57), and our expression profiling suggests that Lipin1 is key to promoting phospholipid formation. Chpt1 is the final step in PTdCho synthesis and is significantly up-regulated. Thus, CPT is not just a marker of ER membrane expansion but may also be a critical factor in the phospholipid response to LPS. However, increased CPT expression alone is not sufficient to drive PTdCho synthesis in other systems (29). The similarity in the gene expression patterns of Lipin1 and CPT suggests that they may be regulated coordinately. XBP-1(S) is a key transcription factor required for plasma cell development (8, 9), but because the genes involved in lipid metabolism are temporally downstream from the proximal differentiation stimulus, it seems most likely that the connection between XBP-1(S) and the biosynthetic genes is indirect, and the transcriptional elements controlling the expression of these key genes warrant further investigation.

There is increased transcription of genes in the de novo biosynthetic pathway that yields the FAs destined for bulk phospholipid and for CCT activation, starting with FA synthase, followed by the genes responsible for acyl-CoA formation, acyl chain elongation, and desaturation. The stimulation of expression of these genes by LPS is consistent with the phospholipid compositional data (Fig. 8) and suggests that the differentiating cells rely mostly on endogenously produced FA to produce PTdCho, the major membrane phospholipid, as well as a portion of the PTdEtn. The Elolv6 transcript level is elevated considerably from a low initial level, marking this enzyme as a key step in promoting the elongation of the 16-carbon fatty acid produced by FA synthase to the 18-carbon fatty acids preferred by the stearoyl-CoA desaturases. Acyl-CoA desaturase (Scd2 and Fads2) gene expression is regulated primarily as an adaptation to in vitro culture. The enhanced expression of the Acsl3 gene encoding an isoform of the acyl-CoA synthetase suggests that this isoform is involved in producing acyl-CoAs for PTdOH formation rather than degradation.

CCT is the most downstream constriction point in PTdCho synthesis, and the regulation of this key step is an important exception to the genetic program outlined above. CCT transcript levels are not elevated in LPS-stimulated CH12 B-cells, although the CK and CPT genes are. Increased CCT expression
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has been implicated in neuronal differentiation (13, 15), but this is not the case in B-cell differentiation. These findings suggest that the constitutive level of CCTα transcripts in CH12 cells is sufficient to support ER expansion upon LPS stimulation. However, two other mechanisms operate to enhance the flux of choline through the CCT step. CCTα protein content increases during differentiation because of a decrease in protein turnover, and CCTα exhibits increased affinity for B-cell membrane lipids following LPS addition, thus activating the enzyme. The activation and affinity for membrane lipids correlate with increased in vivo activity. The increase in DAG and FA production correlates with the stimulation of CCTα activity by CH12 cell lipids (10). These lipids promote an increase in membrane elastic stress, a key membrane property regulating CCT activity (24).

The complex program of genetic and biochemical regulation revealed by these data provide the framework for understanding how B-lymphocytes metabolically activate phospholipid synthesis to support intracellular membrane biogenesis for the specialized overproduction of Ig proteins. Both LPS and IL-5 stimulate similar morphological and biological changes in CH12 B-cells and elicit similar molecular responses (Fig. 10), indicating the generality of the program. There is no single step that is key to the process, but rather the cooperative up-regulation of a handful of key enzymes is required to accelerate PtdCho formation. In the context of LPS-driven B-cell differentiation, Elovl6, Lpin1, and Chpt1 are likely to be critical lipid metabolic genes targeted for transcriptional activation. In contrast, the rate-limiting enzyme in PtdCho biosynthesis, CCTα, is biochemically regulated by indirect means that are the result of genetic activation of upstream components.

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