EFFECT OF LIPOPOLYSACCHARIDE STIMULATION ON THE TRANSCRIPTION AND TRANSLATION OF MESSENGER RNA FOR CELL SURFACE IMMUNOGLOBULIN M*

By DOROTHY YUAN and PHILIP W. TUCKER

Department of Microbiology, The University of Texas Southwestern Medical School, Dallas, Texas

Messenger RNA that code for the μ chain of secreted (s) and cell surface (m) immunoglobulin M (IgM) differ in size (μ_s mRNA are 2.4 kilobases [kb]; μ_m mRNA are 2.7 kb) and in sequence at their 3′ termini (1–4). These sequences predict two different carboxy terminal amino acid sequences and account for peptide (5–7) as well as hydrophobicity (8, 9) differences that have been shown for the two molecules. The two RNA are, however, transcribed from a single Cμ gene (10) by alternative recognition of different polyadenylation sites. The mechanism for the regulation of the level of the two mRNA within different B cell populations is not known, although myeloma and hybridoma cells that are actively secreting Ig (1, 2) as well as fetal liver hybridomas (11) that synthesize only cytoplasmic μ chains contain more of the 2.4 kb μ_s mRNA, whereas lymphoma cells (1, 2) and Abelson virus-transformed pre-B cell lines (12) contain approximately equal amounts of both RNA. In an attempt to understand the regulation of the transcription and translation of these two mRNA as a cell is stimulated from a quiescent state to that of active secretion, we examined changes in the amount of both μ-specific mRNA and polypeptide in a B cell tumor line, BCL_1 (13), before and after induction to Ig synthesis by a mitogen.

We chose the in vivo line of BCL_1 for these studies because these cells are closely analogous to the relatively immature B lymphocyte in that they express a high IgM/IgD ratio and do not secrete IgM (14, 15). They can, however, be activated by mitogens such as lipopolysaccharides (LPS)[1] and will secrete 19s IgM that has the same idiotype as the cell surface IgM (15, 16). The in vivo line of BCL_1, therefore, differs from the line that has been adapted to grow in vitro (17) in that the latter secretes IgM at a low level without additional stimulation and has been shown to contain μ_m mRNA and μ_s mRNA in approximately equal amounts (1). In contrast, in the present studies we show that the μ chain mRNA made by the in vivo cell line is predominantly 2.7 kb in size, suggesting that the cells are not in an activated state in regard to Ig secretion. We are, therefore, able to analyze regulation of μ mRNA synthesis in this cell line. Our studies document that, upon stimulation to secretion

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[1] *Abbreviations used in this paper:* BSS, balanced salt solution; C_μ, μ constant region; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; Id, idiotype; kb, kilobase; kd, kilodalton; LPS, lipopolysaccharide, MEM minimal essential medium; mIgM, cell surface IgM; μ_m, μ chain of cell surface IgM; μ_s, μ chain of secreted IgM; mRNA, messenger RNA; NP-40, Nonidet-P40; PBS, phosphate-buffered saline; RAλ, rabbit anti-mouse λ; RAμ, rabbit anti-mouse μ; rRNA, ribosomal ribonucleic acid; SDS-PAGE sodium dodecyl sulfate-polyacrylamide gel electrophoresis; sIgM, secreted IgM; SSC, 0.15 M NaCl, 0.015 M Na citrate; TCA, trichloroacetic acid; 2-ME, 2-mercaptoethanol.

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with LPS, both species of mRNA for μ chains increase in amount, although the rate of polypeptide synthesis of the μ chain of mIgM in the activated cells is decreased by at least twofold. Regulation of synthesis of the μ chain for secreted vs. cell surface IgM may therefore occur at both the transcriptional and translational level.

Materials and Methods

Cells. BCL_1 cells were maintained by serial passage in BALB/c mice (Cumberland Farms, Clinton, TN). In all experiments, BCL_1 cells were isolated from peripheral blood of tumor-bearing mice (3 mo past inoculation) by means of sedimentation through Isolymph (Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, NY). An average of 10^8 leukocytes/ml can be obtained from tumor-bearing mice, 70–90% of which have been shown to bear Ig with λ-light chain and the BCL_1 idiotype (18). Very few of the Id^- cells are B lymphocytes but consist of Thy-1^+ T cell blasts and polymorphonuclear cells (M. Muirhead, unpublished observations).

Cell Culture. Washed leukocytes were resuspended at 10^6 cells/ml in RPMI medium supplemented with penicillin-streptomycin, 10% fetal calf serum (FCS), glutamine, sodium pyruvate, and 5×10^{-5} M 2-mercaptoethanol (2-ME). All media and supplements were purchased from Gibco Laboratories, Grand Island Biological Co., Grand Island, NY. Where indicated, LPS (*S. typhosa*) (Difco Laboratories, Detroit, MI) was added at 50 μg/ml. Cells were cultured in 150-cm^2 flasks (Corning Glass Works, Science Products Div., Corning, NY).

RNA Isolation. Cultured cells were harvested by centrifugation at 1,500 rpm for 10 min, washed once with balanced salt solution (BSS), and extracted for RNA by one of two methods: (*a*) cells were resuspended at 2×10^7 cells/ml in lysing buffer containing 0.05 M Tris, pH 7.2, 0.005 M $MgCl_2$, 0.0105 M LiCl, 0.005 M uridyl-vanadyl complexes (P. Tucker, unpublished results), and 0.5 mg/ml heparin (Sigma Chemical Co., St. Louis, MO), lysed by the addition of 0.05% Nonidet P-40 (NP-40) (Gallard-Schlesinger Chemical Mfg. Corp.), and centrifuged at 10,000 rpm for 10 min to remove nuclei. Equal volumes of Tris-saturated phenol (J. T. Baker Chemical Co., Phillipsburg, NJ) (pH 7.0) were added to the lysate and heated to 65°C for 5 min. 1/3 vol of chloroform:isoamyl alcohol (95:1) (Mallinckrodt Inc., Science Products Div., St. Louis, MO) was then added and the phases separated by centrifugation. The aqueous phase was reextracted two more times with phenol:chloroform:isoamyl alcohol and precipitated with 3× volume of ethanol. (*b*) RNA was prepared by a method modified from published procedures of Auffray and Rougeon (19). Cells were resuspended at 4×10^7 cells/ml in 3 M LiCl, 6 M urea, and disrupted in a glass homogenizer. The homogenate was sonicated for 2 min (to shear DNA) and kept at 0°C for 16 h. Precipitated RNA was collected by centrifugation at 16,000 rpm for 40 min, extracted with chloroform:isoamyl alcohol (24:1), and reprecipitated with ethanol.

Membrane-bound polysomal RNA was prepared by resuspending cells in a buffer containing 0.05 M Tris, pH 7.2, 0.005 M $MgCl_2$, 0.025 M NaCl, 0.2 M sucrose, 0.005 M uridyl-vanadyl complexes, and 0.5 mg/ml heparin. After lysis by the addition of 0.05% NP-40 and removal of nuclei by centrifugation, the lysate was layered over a step gradient containing 2 ml of 2.5 M sucrose, 4 ml of 1 M sucrose, and 0.6 ml of 0.5 M sucrose and centrifuged at 200,000 *g* for 90 min at 4°C in an SW 41 rotor. The membrane-bound polysomal band was collected at the 2.5 M sucrose interface and extracted with phenol.

DNA Labeling. A μ-specific cDNA plasmid probe, pμ(3741)[9] (20), which contains the coding sequences for the majority of the μ-constant region, was labeled by nick-translation to a specific activity of $\sim 10^8$ cpm/μg, as described previously (21).

RNA Gels and Hybridization. Samples were dissolved in 6% formaldehyde (Mallinckrodt Chem Inc., Science Products Div.), 50% formamide (Sigma Chemical Co.), and 0.02 M phosphate buffer (pH 7.1), denatured by heating for 2 min at 90°C, quick chilled, and electrophoresed in 2% agarose gels buffered in 0.02 M phosphate containing 6% formaldehyde. Gels were washed with hot water before staining in ethidium bromide to locate positions of 28S and 18S rRNA. The RNA was subsequently transferred to nitrocellulose filters, dried, and hybridized to 10^6 cpm/ml of ^{32}P-labeled μ probe in Denhardt's solution containing 5 × SSC (SSC = 0.15 M NaCl, 0.015 M Na citrate) and 50% deionized formamide at 40°C, as previously described (22). Filters were washed and exposed to x-ray film at −70°C (Kodak X-Omatic;

Eastman Kodak Co., Rochester, NY) for 1–7 d.

Cell Surface Labeling. Cultured BCL_1 cells were washed and radioiodinated as described previously (23) at 2.5×10^7 viable cells per ml of phosphate-buffered saline, pH 7.3 (PBS), containing 400 μg lactoperoxidase (Sigma Chemical Co.), 2 mCi of $Na^{125}I$ (Amersham Corp., Arlington Heights, IL), and 25 μl of 0.03% H_2O_2 added at 5-min intervals. The reaction was terminated at 10 min by the addition of excess PBS.

Biosynthetic Labeling. 1×10^8 washed BCL_1 cells were resuspended at 2×10^6 cells/ml in minimal essential medium (MEM) (Gibco Laboratories, Grand Island Biological Co.) supplemented with vitamins, glutamine, antibiotics, 5% FCS, and essential (depleted of methionine) and nonessential amino acids containing 1 mCi of [^{35}S]methionine (1,245 Ci/mmole; Amersham Corp.).

Immunoprecipitation and Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis. Labeled cells were washed twice in PBS, lysed in NP-40 at 2×10^7 cells/ml, and centrifuged at 3,000 rpm for 15 min to remove nuclear material and debris. FCS (50 μl/ml) and phenyl methyl sulfonyl fluoride (1 mM) (Calbiochem Behring Corp., American Hoechst Corp., San Diego, CA) were added to the lysate to minimize proteolysis. Cell lysates and secretions were treated with rabbit anti-mouse μ (RAμ) (24) or rabbit anti-mouse λ (RAλ) (16), and the immune complexes were adsorbed to an excess of *S. aureus* (Cowans I) (25). The bacterial pellets were washed and the immune complexes were eluted by boiling for 1 min in 0.1 M Tris, pH 8.6, containing 8 M urea and 0.2% SDS. The extracts were reduced (0.2 M 2-mercaptoethanol (2ME) and analyzed in either 7.5% polyacrylamide disc gels containing SDS or 10% slab gels according to the method of Laemmli (26). Gels were fixed in 10% trichloroacetic acid (TCA), 10% acetic acid, and 30% methanol, dried, and exposed to x-ray film at −70°C for 2–7 d.

Gel Filtration in NP-40. NP-40 lysates of [^{35}S]methionine-labeled cells, concentrated secretions, or ^{125}I-labeled myeloma IgG reconstituted with 0.5% NP-40 were loaded on a Sepharose 6B (Sigma Chemical Co.) column (90 × 1.5 cm) equilibrated with 0.5% NP-40, 0.01 M Tris-HCl, pH 8.0, 1 mM EDTA, and 0.02% NaN_3, as described by Vassalli et al. (9). 2-ml fractions collected from the column were counted and pooled as shown. IgG from each pool was bound to *S. aureus*, eluted, and analyzed by SDS-PAGE. IgM from each pool was isolated by binding to RAμ and *S. aureus*.

Size Distribution of LPS-stimulated BCL_1 Cells. Cells cultured for 2 d in the presence or absence of LPS were sedimented through 30% Percoll (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Uppsala, Sweden) to eliminate dead cells. The size distribution of each culture was determined by light scatter analysis in the fluorescence-activated cell sorter (FACS) (B-D FACS Systems, Becton, Dickinson & Co., Sunnyvale, CA). A laser light of 488 nm was used at an intensity of 300 mW and scatter gain of 2.

Results

Effect of LPS Stimulation of BCL_1 Cells on the Expression of mRNA for the μ Chain of Cell Surface and Secreted IgM. Peripheral blood lymphocytes from BCL_1 tumor-bearing BALB/c mice were cultured with or without LPS in RPMI supplemented as described. Cells were harvested from the cultures after 3–5 d of incubation and lysed. RNA was isolated from the nuclear-free supernatant. Optical density determination of total RNA recovered indicated that more RNA is usually isolated from LPS-stimulated cells (1.5–2-fold). However, equal amounts of RNA were electrophoresed in 2% agarose gels in the presence of formaldehyde. The intensity of staining by ethidium bromide of the 28s and 18s rRNA was approximately the same for both cultures (data not shown), showing that equal quantities of total RNA from both cultures were analyzed. RNA was transferred to nitrocellulose filter paper and hybridized with [^{32}P]nick-translated μ-specific cDNA probe. As can be seen in Fig. 1 (lanes A1 and B1), BCL_1 cells cultured without LPS contain predominantly one species of mRNA (2.7 kb) that hybridizes with the μ-specific probe. This 2.7 kb RNA has been shown

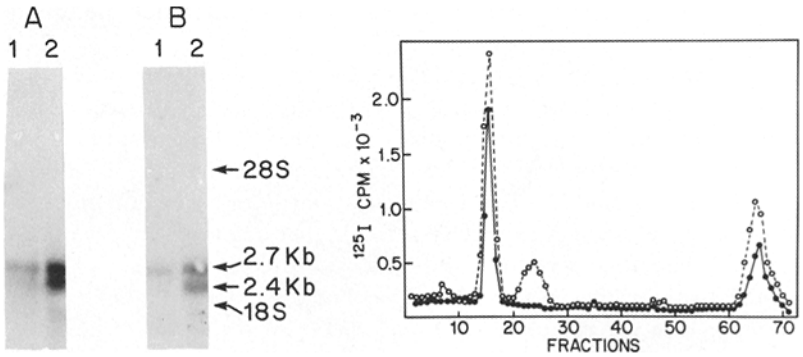

FIG. 1. (left) The effect of LPS stimulation on amounts of RNA specific for the μ chain of cell surface and secreted IgM. PBL from BCL_1 tumor-bearing mice were cultured for 3 d (A) or 5 (B) d in the presence (2) or absence (1) of LPS. RNA was extracted from A cultures by method 1 and from B cultures by method 2, as described in Materials and Methods. 60 μg of RNA was loaded in channel A1 and A2, while 25 μg of RNA was loaded in channels B1 and B2. Positions of 28s and 18s ribosomal RNA were determined by staining gels with ethidium bromide. RNA separated by agarose gel electrophoresis was transferred to nitrocellulose and hybridized with ^{32}P-labeled μ-specific probe.

FIG. 2. (right) Effect of LPS stimulation on surface Ig as detected by surface iodination. 2×10^7 PBL cultured for 4 d in the presence (●) or (⊖) absence of LPS were radioiodinated as described in Materials and Methods. The cells were lysed in NP-40, the Ig isolated by complexing with RAλ and *S. aureus*, and analyzed by SDS-PAGE under reducing conditions. Immunoprecipitation with rabbit anti-μ serum and rabbit anti-δ serum (not shown) shows the peaks migrating at fractions 17, 23, and 65 to be μ, δ, and L chains, respectively.

to encode μ chains for cell surface IgM (μ_m mRNA) (1, 2). 3–5 d after LPS stimulation, BCL_1 cells synthesize approximately equimolar amounts of two species of RNA, a 2.4 kb species, in addition to the 2.7 kb μ_m mRNA (lanes A2 and B2). The former has been shown to encode the μ chain for secreted IgM (μ_s mRNA) (1, 2). Densitometric scans of the autoradiographs of Fig. 1 determined that the amount of 2.7 kb μ_m mRNA is increased by 3–4-fold, whereas the 2.4 kb μ_s mRNA is increased 6–12-fold in LPS-stimulated cells.

The increase in μ-specific RNA is probably not an artifact of the isolation procedure because the same observation was made when RNA was isolated by two entirely different protocols: phenol extraction of nuclear-free lysate (Fig. 1 A) and LiCl-urea precipitation in the presence of DNA (Fig. 1 B, and see Materials and Methods). Furthermore, RNA preparations were not further fractionated by procedures such as oligo(dT) chromatography to insure that differential losses did not occur.

Expression of Cell Surface IgM in LPS-stimulated Cells. The increase of μ_s mRNA in LPS-stimulated cells was not unexpected because LPS induces BCL_1 cells to secrete substantial amounts of 19s IgM (14, 15). To determine whether the increase in μ_m mRNA is also correlated with an increase in expression of cell surface IgM, BCL_1 cells cultured with or without LPS for 4 d were surface labeled by lactoperoxidase-catalyzed iodination. Equal numbers of TCA precipitable counts from each nuclear-free cell lysate were complexed with RAλ and bound to *S. aureus*. The immune complexes were subsequently eluted, reduced with 2-ME, and analyzed by SDS-PAGE. The disc gels were fractionated by a gel slicer and counted. It was found that the number of counts associated with the μ chain of cell surface IgM of LPS-stimulated cells was only 61% of that of the μ chain from cell surface IgM of nonstimulated cells

(Fig. 2). As has been shown by others (27), the amount of iodinatable IgD decreases more drastically after LPS stimulation.

Rate of Synthesis of Cell Surface IgM in LPS-stimulated Cells. Cell surface expression of IgM in LPS-stimulated BCL_1 cells apparently decreases, as detected by surface iodination, despite an increase in amount in μ_m mRNA. This could be because of a number of reasons: (*a*) masking of cell surface components making IgM of LPS-stimulated cells less available for surface iodination; (*b*) a lower rate of synthesis of μ chain destined for cell surface expression; (*c*) a higher degradation rate of cell surface IgM; or (*d*) a lower efficiency of translation for μ_m mRNA. We designed experiments to distinguish between the above possibilities. First, we biosynthetically labeled both LPS-stimulated and nonstimulated cells and determined the rate of label accumulation in the μ chain of cell surface IgM in each case. 3 d after initiation of culture, cells were resuspended in labeling medium containing [^{35}S]methionine. Equal aliquots were removed at various intervals after the initiation of labeling, lysed, the nuclear-free supernatants complexed to RAμ, and isolated by binding to *S. aureus*. Immune complexes were subsequently eluted, reduced with 2-ME, and analyzed by slab-gel electrophoresis. It can be seen in Fig. 3 that two heavy chain bands, 82 kd and 76 kd, are present in all cell lysates (lanes 1–5). We have shown previously (5) that the 82 kd

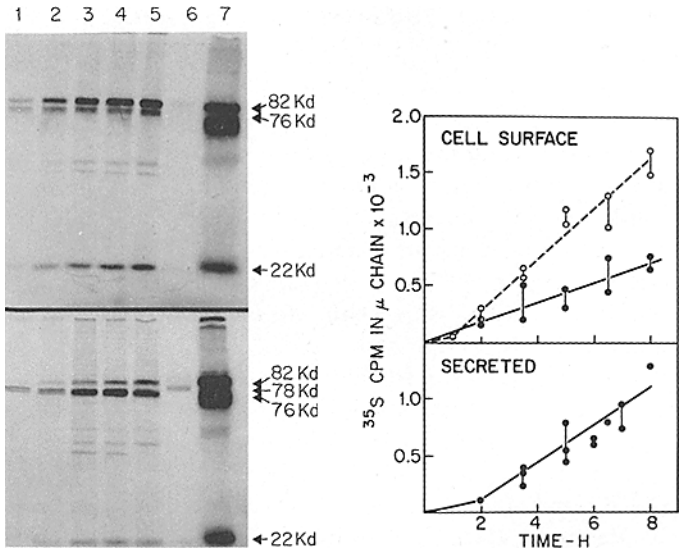

FIG. 3. (left) Effect of LPS on the kinetics of label incorporation into cell-associated IgM. PBL cultured for 3 d in the presence (bottom panel) or absence (top panel) of LPS were biosynthetically labeled with [^{35}S]methionine. Aliquots of labeled cells (5×10^6 cells) were removed at 2, 3.5, 5, 6.5, and 8 h (lanes 1–5), lysed, immunoprecipitated, and analyzed by SDS-PAGE under reducing conditions. The labeling medium of each sample was immunoprecipitated and analyzed similarly. An aliquot of the precipitate from the 8-h sample of each culture was loaded in lane 6. Lane 7 shows the Ig profile of surface iodinated normal lymphocytes.

FIG. 4. (right) Kinetics of label incorporation into μ chain of cell surface (top panel) and secreted (bottom panel) IgM. Top panel: the 82 kd band from SDS-PAGE slab gels of two experiments performed as in Fig. 3 were cut out and counted in the liquid scintillation counter. (⊖), minus LPS; ●, plus LPS. *Bottom panel:* the media of LPS-stimulated cells labeled for various periods were immunoprecipitated and analyzed by SDS-PAGE. The 78 kd μ chain bands were cut out and counted as above. The data represent values derived from two experiments. Some of the points are repeat determinations of the same sample.

band represents the μ chain of biosynthetically labeled cell surface IgM. Co-migration with ^{125}I-surface-labeled IgM confirms this identification (lane 7). The 76 kd band probably consists of partially glycosylated intracellular μ chains destined both for membrane insertion and secretion, as has been observed in normal B lymphocytes (28–30); such μ chains are absent in lysates of cells labeled after treatment with tunicamycin, which inhibits glycosylation (28–31). Immunoprecipitation with RAμ of the labeling medium from LPS-stimulated cells results in the isolation of secreted IgM. Lane 6, bottom panel, shows that the μ chain of this IgM has an apparent molecular weight of 78 kd, whereas precipitation of labeling medium of nonstimulated cells failed to demonstrate any secreted IgM (lane 6, top panel).

The amount of label in each cell surface μ chain band was determined more accurately by cutting out the bands and counting in a liquid scintillation system (Fig. 4, top panel). It is clear that the rate of label accumulation in the cell surface μ chain of LPS-stimulated cells is <50% of that in nonstimulated cells, closely corresponding to our finding from surface iodinated cells. The rate of label accumulation in the μ chain of IgM secreted by the same cells is, on the other hand, ~50% higher than that of the μ chain of cell surface IgM (Fig. 4, bottom panel). The apparent decrease in label accumulation could be the result of an increased degradation rate of cell surface IgM in LPS-stimulated cells. We, therefore, labeled both cultures for only 1 h and "chased" the label by resuspending the cells in medium containing unlabeled methionine. IgM was subsequently precipitated from aliquots at various times after incubation and analyzed by SDS-PAGE (Fig. 5). The amount of cell surface μ chain in both LPS-stimulated (right panel) and nonstimulated (left panel) cells was found to remain constant up to 7 h after the "chase" (lane 4) and decreased to ~50% at 24 h (lane 5).

Although the radioactivity associated with the μ chain of cell surface IgM in either LPS-stimulated or nonstimulated cells does not decrease significantly, the fate of the intracellular, 76 kd band differs for the two cell cultures. The 76 kd band in nonstimulated cultures has a relatively fast turnover rate, while part of the 76 kd band in LPS-stimulated cultures persists even after overnight incubation. Because the number of counts associated with IgM in the culture supernatant of LPS-stimulated cells plateaus at 3 h after initiation of chase (data not shown), the radioactivity that remains associated with the 76 kd intracellular band does not seem to represent an immediate precursor of secreted IgM.

Detergent-binding Characteristics of Biosynthetically Labeled IgM. It is possible that part of the 76 kd band could be coded for by the "excess" μ_m mRNA and, thus, may have a hydrophobic region (2) distinguishing it from the μ chains encoded by μ_s mRNA. We, therefore, determined the detergent-binding ability of the 76 kd band under native conditions by gel filtration through a sizing column in the presence of NP-40. The gel filtration properties of molecules with exposed hydrophobic regions have been found to be analomous in such columns (9; and D. Yuan and E. S. Vitetta, manuscript submitted for publication), in that they are eluted faster than nondetergent-binding molecules of similar size. A lysate from LPS-stimulated cells labeled for 1 h and chased for 7 h in cold methionine was loaded on a Sepharose 6B column equilibrated in 0.5% NP-40. Fig. 6 (top panel) shows the profile of total radioactivity recovered from the column. Fractions were pooled as shown, and each bound to RAμ and *S. aureus*. Immunocomplexes were then eluted, reduced, and analyzed by gel electropho-

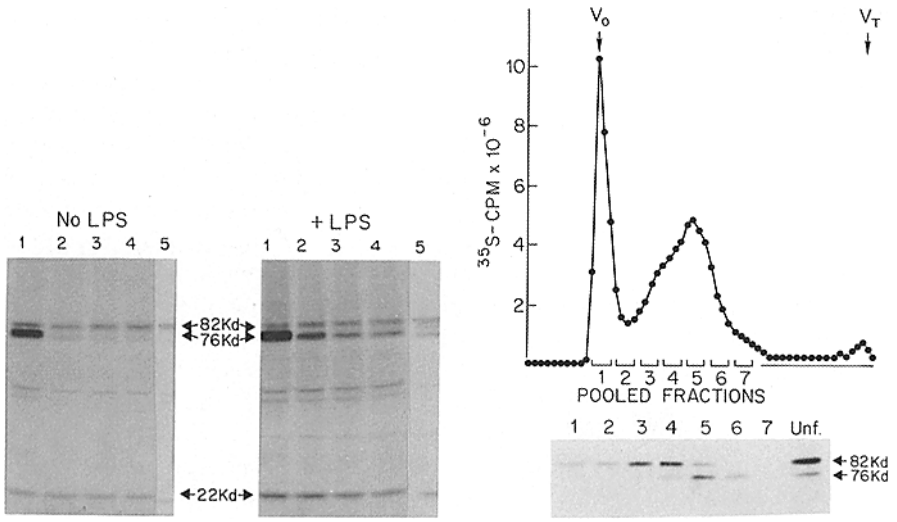

FIG. 5. (left) Comparison of stability of labeled IgM from LPS-stimulated and nonstimulated cells. PBL cultured for 3 d with (right hand panel) or without (left hand panel) LPS were biosynthetically labeled for 1 h with [^{35}S]methionine and resuspended in unlabeled medium. Aliquots were removed immediately or at 3, 5, 7, and 24 h (lanes 1–5) after resuspension in unlabeled medium. IgM was isolated with RAμ and *S. aureus* and analyzed by SDS-PAGE.

FIG. 6. (right) Gel filtration characteristics of IgM of LPS-stimulated cells. PBL stimulated by LPS for 3 d and labeled and chased for 8 h, as described for Fig. 5, were lysed. After overnight dialysis, the nuclear-free lysate was loaded on a Sepharose 6B column equilibrated in 0.5% NP-40. 20-μl aliquots of each 2-ml fraction were counted before they were pooled, as shown, and immunoprecipitated with RAμ and *S. aureus*. The μ chain region of SDS-PAGE analysis of each fraction is depicted.

resis. As can be seen in the bottom panel, the majority of cell surface IgM (containing 82 kd μ chain) was eluted in fractions 3 and 4, whereas the peak of IgM containing the 76 kd μ band was eluted in fraction 5. Although not shown, light chain bands were present in all fractions. Therefore, the 76 kd intracellular μ chain, which has a long half-life in LPS-stimulated cells, does not bind sufficient detergent to change its gel filtration properties and is most probably encoded by μ_s mRNA and not μ_m mRNA. Control experiments showed that ^{125}I-labeled cell surface IgM (8S) eluted in fractions 1–4 as a result of the binding of detergent, while in the same column, ^{125}I-labeled myeloma IgG (7S) eluted in fraction 5 (not shown).

Proportions of μ_m vs. μ_s mRNA in Polysomes. Because the LPS-stimulated increase of μ_m mRNA does not seem to be reflected at the protein synthetic level, it is possible that, although synthesized, some of the mRNA is not bound to polyribosomes. Because the μ chains for both sIgM and mIgM are synthesized on membrane-bound polyribosomes, a decrease in binding would account for the decrease in translation of μ_m mRNA. Polysomal RNA was, therefore, isolated from BCL_1 cells cultured with or without LPS for 3 d. As shown in Fig. 7, membrane-bound polysomal RNA that hybridizes with the μ-specific probe displays the same profile as total RNA in that both μ_s and μ_m mRNA are enriched in LPS-stimulated cells. Therefore, the additional μ_m mRNA seem to bind to polyribosomes with similar efficiency as μ_s mRNA.

Proportion of Cells Stimulated by LPS. Although BCL_1 is a monoclonal population (16), the effect of LPS stimulation on the cells is not uniform in that only ~10% of the

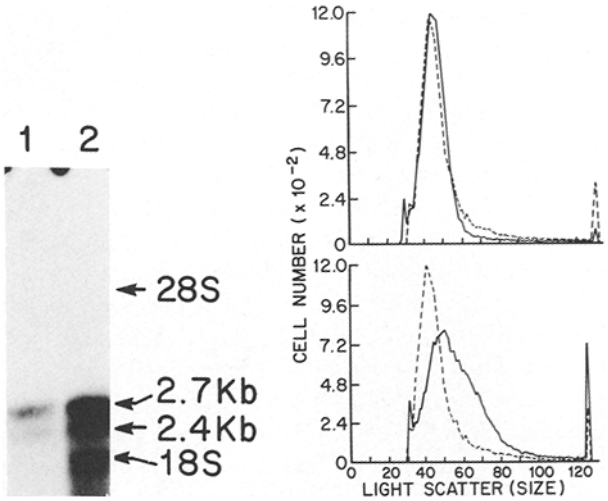

FIG. 7. (left) Effect of LPS on μ-specific polysomal RNA. Cells cultured in the presence (lane 2) or absence (lane 1) of LPS were lysed and the polysomal RNA prepared from the nuclear-free lysate. 60 μg of RNA from each culture was separated by agarose gel electrophoresis, transferred to nitrocellulose, and hybridized with a ^{32}P-labeled μ chain-specific probe.

FIG. 8. (right) Change in size distribution of BCL_1 cells after LPS stimulation. PBL cultured for 1 d (- - -) or 2 d (——) with (lower panel) or without (upper panel) LPS were analyzed on the FACS. Cells falling below channel 33 represent erythrocytes and were gated out. Scatter signals were standardized daily by using glutaraldehyde-fixed chicken erythrocytes.

cells are induced to plaque-forming cells (32). Cytoplasmic staining of LPS-stimulated cells (D. Yuan and K. Krolick, unpublished observations) showed bright staining in only 30% of the population. However, among the cells staining with lower intensity, we could not distinguish those cells that are secreting Ig at a lower rate from those that are not secreting but contain cytoplasmic Ig destined for membrane insertion. We also examined the size distribution of BCL_1 cells cultured in the presence or absence of LPS. Fig. 8 shows that, 2 d after stimulation, virtually all of the cells increased in size, suggesting that all of the cells have been stimulated by LPS to proceed from G_0 to G_1 phase of the cell cycle, although we cannot determine the exact fraction of those that go on to initiate secretion of Ig.

Discussion

The central finding in this paper is that stimulation of BCL_1 cells by LPS results in at least two molecular events: (*a*) an apparent increase in the transcription rate of both μ_s and μ_m mRNA; and (*b*) the induction of mRNA for μ_s. Our results also suggest that additional regulatory events may occur, which decreases the translation of μ_m RNA in stimulated cells.

Because LPS induces BCL_1 cells to secrete IgM, it was not unexpected to find this induction to be correlated with an increase in the amount of mRNA for the secreted μ chain. An increase in mRNA for κ chains has been previously documented in both antigen and LPS-stimulated normal B lymphocytes (33, 34). In addition, a substantial increase in κ chain RNA occurs after LPS stimulation of the pre-B cell-like lymphoma 70Z/3 (35). In BCL_1 cells, however, the amount of μ_m mRNA is also increased concomitantly with μ_s mRNA. The extent of the increase is not as great for μ_m mRNA

as for μ_s mRNA because the former is already present in higher quantities in unstimulated cells. In contrast to the μ chain of secreted IgM, the increase in μ_m mRNA is not accompanied by an increase in the synthesis of the μ chain of membrane IgM, as reflected by the amount of surface μ available for iodination. A decrease in surface IgM of WEHI 231 tumor cells after LPS stimulation has been described (36), although earlier data obtained from normal cells (37) suggested an increase in surface IgM of LPS-stimulated normal cells after a transient phase (~12 h) of decrease. We have carefully investigated this question in BCL_1 cells and have shown that the decrease of the surface μ chain after LPS stimulation is due to a decrease in the rate of biosynthesis of the molecule. This conclusion is based on measuring the rate of accumulation of label into the μ chain of cell surface IgM over a period of 8 h. The rate of accumulation of label into a protein is determined primarily by three factors: (*a*) the rate of synthesis of the protein; (*b*) the rate of degradation; and (*c*) the specific activity of the amino acid pool. We have shown, by a "pulse-chase" experiment, that the rate of degradation of cell surface IgM is similar in both cultures. The specific activity of the amino acid pool for both cultures is probably also similar because the kinetics of incorporation of radioactivity into total TCA precipitable proteins were identical (data not shown). Therefore, the rate of accumulation of label into the cell surface μ chain most probably reflects the rate of synthesis of the protein.

Because the rate of cell surface Ig synthesis is decreased, it is possible that the increased amount of μ_m mRNA may be used to code for a cytoplasmic μ chain that is not exteriorized but is rapidly degraded. However, our pulse-chase experiment (Fig. 5) shows that the intracellular μ chains of LPS-stimulated cells actually appear to have a longer half-life than those of nonstimulated cells. This species of 76 kd μ chain, which cannot be chased into the medium, may be related to the 70 kd μ chain, described by Sidman (30), that is present in IgM-secreting hybridoma cells. The latter has been shown to have a longer-half-life, and it also has no exposed detergent-binding site. The origin and fate of this μ chain species is not known. It might represent improperly glycosylated μ chains that cannot be secreted but remain sequestered within the cell cytoplasm. Totally nonglycosylated IgM synthesized under conditions of tunicamycin inhibition has been shown to remain associated with BCL_1 cells and is not secreted (31). It is interesting to note that, although the 76 kd band in nonstimulated cells is more rapidly turned over (Fig. 5), it is not chased into cell surface IgM because radioactivity associated with the fully processed 82 kd μ band does not increase substantially with time after the chase. Gel filtration analysis in NP-40 (data not shown) indicates that most of the 76 kd band in nonstimulated cells also does not bind detergent, suggesting that these are μ chains synthesized from μ_s mRNA that are not secreted but degraded. Experiments are in progress to examine this point further.

Although we have shown that virtually all of the cells have initiated the differentiation process by an increase in size after LPS stimulation, it is possible that only a portion of the cells are stimulated to secrete because the rate of accumulation of label in μ_s in LPS-stimulated cells (Fig. 4) is not as high as one would expect from the observed increase in μ_s mRNA. If the decrease in synthesis of μ_m occurs only in fully differentiated Ig-secreting cells, the observed discrepancy between the amount of μ_m mRNA and the synthetic rate of cell surface μ in LPS-stimulated cells would be an underestimate.

Our results differ from those that have been obtained in the in vitro BCL_1 cell line (S. Strober, personal communication), where no change was observed in the abundance of μ-specific mRNA after LPS stimulation. However, because the in vitro cell line already secretes IgM from a significant pool of μ_s mRNA available before stimulation, the effect of LPS on these cells might differ from that on a less-activated cell such as the in vivo line.

In conclusion, after activation by LPS, the amount of mRNA for both secreted and cell surface μ chain in BCL_1 cells is increased concomitantly. The μ_s mRNA is used to translate the increased amount of Ig secreted by the cell. The decrease in the rate of synthesis of μ_m, however, suggests that there must exist an additional regulatory step that decreases the extent of translation of the mRNA for μ_m. This type of regulation probably differs from many other cases of post-transcriptional control, which has been documented in eucaryotic cells (38–41). For example, the differential translation of α and β-globin mRNA in erythrocytes (38) has been attributed to differences in binding sequence for initiation factor eIF-2 and the 5′ terminal cap structure. In contrast, μ_s mRNA and μ_m mRNA have identical sequences at their 5′ region, therefore, they should not differ in their ability to form initiation complexes with ribosomes. Moreover, the two mRNA code for identical leader sequences, and, therefore, the ability of each nascent polypeptide chain, μ_m or μ_s, to insert into the membrane of the endoplasmic reticulum should also not differ. This prediction is confirmed by our analysis of the μ-specific mRNA content of membrane-bound polyribosomes. The regulatory step must, therefore, occur sometime between the binding of the polyribosomes to the endoplasmic reticulum and the completion of the μ chain. The mechanism behind this type of translational control might be similar to that used by heat-shocked drosophila cells in which preexisting mRNA molecules synthesized before heat shock remain bound to polyribosomes but are however not translated (42). It should be noted that the translational control that results in the decreased synthesis of μ_m is distinct from the post-translational modification events involved in the fate of partially glycosylated 76 Kd μ chains discussed earlier.

The RNA coding for cell surface μ chain of most myeloma and hybridoma cell lines is much lower than that for secreted μ. Correspondingly, very little surface Ig can be demonstrated in these cells. It is possible, therefore, that BCL_1 cells require an additional differentiation step involving a decrease in synthesis of μ_m mRNA before it can display the typical phenotype of true plasma cells. Alternatively, because the BCL_1 cell line has some characteristics of relatively immature B cells, LPS stimulation might induce its differentiation to a different pathway from that taken by more mature B cells.

Finally, because BCL_1 cells are neoplastic in origin, the possibility does exist that the regulatory pathways described here are not representative of normal B lymphocytes. However, the turnover rate of membrane IgM in BCL_1 cells as well as the pattern of Ig synthesis in both nonstimulated and stimulated cells closely resemble that which has been shown for normal B lymphocytes. It should be noted that we have only measured the total amount of mRNA in these cells before and after LPS stimulation. It is possible that the apparent increase in μ RNA might be because of an increased stability of the message after LPS stimulation. Further experiments are in progress to examine this possibility.

Summary

Analysis of μ-specific mRNA in the B cell tumor line, BCL_1, shows that the cells contain predominantly mRNA for μ chain of membrane-bound immunoglobulin M (IgM) (2.7 kb, μ_m mRNA). Stimulation of the cells to Ig secretion by lipopolysaccharide (LPS) results in a 6–12-fold increase in amount of mRNA for the μ chain of secreted IgM (2.4 kb μ_s mRNA). The increase in μ_s mRNA is accompanied by a 3–4-fold increase in μ_m mRNA. The rate of μ chain synthesis of membrane IgM in LPS-stimulated cells is, however, reduced by at least twofold, suggesting that both transcriptional and translational regulatory events are involved in the induction of B lymphocytes to secretion.

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