CHANGES IN J CHAIN AND μ CHAIN RNA EXPRESSION AS A FUNCTION OF B CELL DIFFERENTIATION

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An immunocompetent B lymphocyte can be triggered by the appropriate antigen and lymphokine signals to differentiate into a pentamer IgM–secreting cell. The process involves a change in the function of the IgM antibody molecule; it is converted from a membrane receptor for antigen to a secreted polymer that effects antigen disposal. This change is known to require (*a*) synthesis of the secreted form of μ heavy chain and (*b*) synthesis of the polymerizing component, the Ig J chain.

Membrane and secreted forms of the μ heavy chain were first distinguished by their different mobilities in polyacrylamide gels (1). Subsequent studies showed that the structural differences are confined to the carboxy-terminal sequence of the chain and correlate with the different functions of the IgM molecule (2, 3). The membrane form has a hydrophobic terminus that anchors the IgM monomer in the B cell membrane whereas the secreted form has a shorter, more hydrophilic terminus that contains the penultimate cysteine residue required for pentamer IgM assembly. The two termini were found to be encoded in exons located 3′ to the Cμ gene and are cotranscribed with the gene (4). Separate μ_m and μ_s mRNAs are then generated from the single gene transcript by differential 3′ cleavage and processing. These data established that the switch in μ chain synthesis during B cell differentiation is regulated at the posttranscriptional level.

The assembly and secretion of pentamer IgM also depends on the synthesis of a second protein, the J chain (5, 6). The J polypeptide serves to initiate IgM polymerization by bridging two IgM monomers through disulfide bonds to the μ_s carboxy termini. The resulting J chain–containing dimer then promotes the disulfide bonding of three additional monomers to complete the pentamer structure. Studies of rabbit and human splenocytes (7, 8) have shown that J chain expression is a direct result of B cell activation. Little or no intracellular J chain could be detected in the unstimulated cell populations. After stimulation, however, large amounts of J chain were observed concomitant with the appearance of secreted IgM in the culture supernatants. Moreover, analyses of J chain mRNA in transformed cell lines showed that J chain–specific sequences were absent from a line representing an unstimulated B cell, but were present in large amounts in a myeloma cell line secreting pentamer IgM (9). These results indicate

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that J chain synthesis is induced during B cell differentiation by transcription of a previously silent gene.

The mechanisms for signaling these posttranscriptional and transcriptional events are not well understood. It is known that B cell differentiation requires multiple membrane stimuli; in addition to antigen, a number of factors of T cell or macrophage origin have been found to participate in the triggering process (10–18). The binding of antigen to the surface IgM receptor is thought to "excite" the B cell and make it responsive to subsequent proliferative and differentiative signals from the lymphokines (19). To elucidate the signaling effects, we have investigated the expression of μ and J chain mRNA as a function of B cell differentiation. The relative amounts of each mRNA were analyzed in cell lines representing progressive stages of differentiation and in normal mouse splenocytes stimulated with bacterial lipopolysaccharide (LPS).[1] From the results of the analyses, it was possible to determine the time course of μ_s and J chain expression and correlate the kinetics with the actions of different membrane stimuli.

Materials and Methods

Cell Lines. MOPC 104E plasmacytomas (μ, λ_1), a gift from Dr. A. Good, University of California, Berkeley, were grown subcutaneously as solid tumors in BALB/c female mice. A subclone of the B lymphoma line WEHI 231E1 (μ, κ) (20), derived by Dr. E. Siden, University of Florida, Gainesville, was maintained in Dulbecco's modified Eagle's medium (DME) containing 10% horse serum (HS) and 5×10^{-5} M 2-mercaptoethanol (2-ME). The hybrid line M × W 231.1a.2 (21), derived from the fusion of WEHI 231 and MPC 11 (λ_{2b}, κ), was obtained from Dr. Raschke and maintained in DME containing 10% HS. The lymphoma line 70Z/3 (μ, κ) (22) was provided by Dr. P. Kincade, Sloan-Kettering Institute, New York, and the lymphoma line K46R (μ, κ) (23), by Dr. R. Asofsky and Dr. K.-J. Kim, National Institutes of Health, Bethesda, MD. Both lymphoma lines were maintained in RPMI 1640 containing 10% fetal calf serum (FCS) and 5×10^{-5} M 2-ME. The L929 mouse fibroblast cells, obtained from Dr. O. Martinez, University of California, Berkeley, and the IgA-secreting myeloma MOPC 315 (α, λ_2), adapted to tissue culture by Dr. E. Siden, were grown in DME containing 10% FCS. All cell lines were incubated at 37°C in an atmosphere of 10% CO_2.

Isolation of the Small Cell Population of Mouse Splenocytes. Spleens were aseptically removed from BALB/c mice and placed in sterile, ice-cold, balanced salt solution (BSS). Single-cell suspensions were prepared by mincing the spleens in BSS and the erythrocytes were depleted by lysis with hemolytic Gey's solution (24). The remaining cells were suspended at a concentration of 1×10^7 cells/ml in BSS containing 0.3% bovine serum albumin (BSA) and size-fractionated by 1 *g* velocity sedimentation (25) in a Sta-Put apparatus (Johns and Co., Toronto). In a typical experiment, 1×10^9 cells, obtained from eight to nine spleens, were allowed to sediment for 5 h at 4°C through a linear gradient formed by the mixture of 120 ml of 0.5% BSA, 1,200 ml of 1% BSA, and 1,200 ml of 2% BSA from three gradient chambers. Fractions of 40 ml were collected and the number of nucleated cells per fraction was monitored with a Coulter counter (Coulter Electronics, Inc., Hialeah, FL). Those fractions containing the small lymphocyte population were pooled and the cells were pelleted by centrifugation and washed once with BSS.

Culture of Small Cell Population of Mouse Splenocytes. Cells obtained from velocity sedimentation were cultured at an initial density of 4×10^6 cells/ml in RPMI 1640

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; BSS, balanced salt solution; DME, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; HS, horse serum; LPS, bacterial lipopolysaccharide; 2-ME, 2-mercaptoethanol; PBS, phosphate-buffered saline; PFC, plaque-forming cell; TRF, T cell–replacing factor.

containing 10% FCS, 1 mM sodium pyruvate, 2 mM L-glutamine, essential and nonessential amino acids, 5×10^{-5} M 2-ME, and 20 μg/ml LPS (*Escherichia coli* lipopolysaccharide; Difco Laboratories, Inc., Detroit, MI). The same lots of FCS and LPS were used for all experiments. The cultures were incubated at 37°C in an atmosphere of 10% CO_2 and viability was monitored by trypan blue dye exclusion.

RNA Isolation. Cytoplasmic RNA was isolated from cell lines by lysing 5×10^8 pelleted cells in 20 ml of ice-cold lysis solution containing 0.15 M KCl, 10 mM $MgCl_2$, 0.5% (wt/vol) Nonidet P-40, 10 mM Tris-HCl, pH 7.4. After the nuclei and cellular debris were removed by centrifugation at 2,000 *g* for 5 min, the supernatant was extracted three times with an equal volume of 1:1 (vol/vol) phenol and chloroform and one additional time with an equal volume of chloroform alone. The RNA was precipitated from solution in 70% ethanol, 0.4 M NaCl at −20°C. Total RNA was prepared from normal and LPS-stimulated lymphocytes by the guanidine hydrochloride extraction procedure (26). When it was necessary to combine cells from more than one velocity sedimentation separation, the cells were pelleted by centrifugation and stored at −20°C. The pellets were then individually lysed in the 8 M guanidine hydrochloride solution and the lysates were combined for the remaining extractions. Poly(A)+ RNA was selected from both the cytoplasmic and total RNA preparations by oligo(dT)-cellulose chromatography (27) and stored as an ethanol precipitate at −20°C.

Identification of Size-fractionated RNA. For the detection of J chain–specific sequences, poly(A)+ RNA was size-fractionated on methylmercury hydroxide, 1.4% agarose gels (28) and the gels were prepared for transfer by a modification of the procedure of Alwine et al. (29). For the detection of μ chain–specific sequences, poly(A)+ RNA was size-fractionated by use of a formamide-fomaldehyde gel system that allows separation of the membrane and secreted forms of μ RNA. 1–10 μg of RNA was dissolved in 10–50 μl of MOPS buffer (0.2 morpholinopropanesulfonic acid, 50 mM sodium acetate, 10 mM EDTA, pH 7.0) containing 50% (vol/vol) formamide and 20% (vol/vol) formaldehyde, and denatured by heating at 60°C for 5 min and cooling on ice for 5 min. After the addition of 1/10 volume of loading dye (50% glycerol and 0.5% bromophenol blue), the samples were electrophoresed horizontally at 40–50 V for 12–15 h in MOPS buffer on a 1.4% agarose gel containing 6% formaldehyde. RNA was transferred from the gels to nitrocellulose (30) and hybridized (31) with an appropriate probe that was ^{32}P-labeled by nick translation (27) to a specific activity of 5×10^7 to 1×10^8 cpm/μg DNA. For analysis of J chain RNA, cloned cDNA (32), containing the coding information for the mature J protein, was used as a probe. For analysis of μ chain RNA, the probes were a 10.5 kilobase (kb) *Eco*RI genomic fragment that includes the sequences for the entire Cμ gene and a 1.3 kb *Hind*III genomic fragment that includes the coding sequences for the Cμ3 and Cμ4 exons. After washing, the hybridized filters were exposed to Kodak XAR-5 x ray film at −70°C through Cronex Lightning-Plus intensifying screens (Dupont Co., Wilmington, DE). The relative intensities of hybridization were quantitated by densitometer scanning.

Measurements of J Chain and IgM Proteins. Cells expressing membrane IgM were labeled by staining with rabbit anti-mouse μ chain IgG and a rhodaminated $(Fab)'_2$ goat anti-rabbit IgG antiserum. The cells were washed, resuspended in FCS, wet mounted, and observed under a Zeiss standard 18 microscope equipped with an Epi IV FL illuminator. Cells secreting Ig were identified by reverse protein A plaque assays (33) in Cunningham chambers.

The levels of intracellular J chain and monomer IgM and the levels of secreted pentamer IgM were quantitated by a liquid phase double antibody radioimmunoassay (34). For the J chain determinations, the antibody reagents consisted of the IgG fraction of goat anti-mouse J chain antiserum and a rabbit anti-goat IgG antiserum. For the IgM determinations, the primary antibody was an IgG fraction of rabbit anti-mouse μ chain and the secondary reagent was a goat anti-rabbit IgG antiserum. The purified proteins used as inhibition standards were labeled with ^{125}I by the use of lactoperoxidase Enzymobeads (Bio-Rad Laboratories, Richmond, CA). Labeled J chain was separated from free $Na^{125}I$ by trichloroacetic acid precipitation; labeled monomer and pentamer IgM were separated

from free label by filtration through a 5-ml column of P-4 resin (Bio-Rad Laboratories). Specific activities averaged 1×10^4 cpm/ng of protein.

The preparation of cell lysates and culture supernatants and the assays for inhibitory activity were performed according to the protocols described by Mather (34) with the following modifications: (*a*) all dilutions of antigens and antisera were made in PBS containing 0.45% Nonidet P-40, 1% BSA, and 0.02% sodium azide; (*b*) test samples were incubated with the appropriate antibody for 1 h at 37°C before the addition of labeled antigen; (*c*) after addition of labeled antigen the mixtures were incubated for 1 h at 37°C and 16–20 h at 4°C and then facilitated.

Results

Analysis of J Chain– and μ Chain–specific RNA in Lymphoid Cell Lines. Murine cell lines assigned to successive stages in B cell differentiation (20–23) were chosen as sources of RNA. These included the lymphoma 70Z/3, which represents a transition stage between a pre-B cell and an immature B cell; the lymphomas WEHI 231 and K46R, which represent immature and mature B cells, respectively; and the MOPC 104E plasmacytoma and M × W 231.1a.2 hybrid cell line, which are characteristic of pentamer IgM secretors. Cytoplasmic RNA was isolated from each cell line and from two control populations, the L929 fibroblast line and an IgA-secreting plasmacytoma, and the poly(A)+ fraction was examined for J chain– and μ chain–specific sequences by the Northern blotting technique.

The analyses showed that changes in J and μ chain RNA expression were confined to a single step in the differentiation pathway, namely the conversion of a mature B lymphocyte to an IgM-secreting cell. In the case of J chain RNA the change was all or none (Fig. 1). No J chain–specific sequences were detected in the lymphoma RNA preparations regardless of the maturation stage represented. The blots were negative after 21 h of exposure, as shown in Fig. 1, as well as after 140 h of exposure (data not shown). In contrast, large amounts of J chain RNA were detected in the Ig-secreting lines. Three species were expressed: a major band of 1.5 kb that corresponds to the mature message and two minor bands of 2.7 and 0.9kb that are of unknown function.

In the case of μ chain RNA, however, the change was relative rather than absolute (Fig. 2 *a*). All cell lines examined were found to express both membrane and secreted forms of μ chain RNA, but lines representative of IgM secretors contained a significantly higher proportion of the secreted form. The extent of the μ_s shift was quantitated by scanning each autoradiograph with a densitometer and determining the percentage of μ_s mRNA from the relative areas under the peaks (Fig. 2 *b*). The results showed that the secreted form constituted 40% of the μ chain mRNA in the lymphoma lines, again regardless of the maturation state represented. By comparison, the secreted form constituted 91 and 72% of the μ chain mRNA in the IgM-secreting lines, MOPC 104E plasmacytoma and M × W hybrid, respectively.

Analyses of Mitogen-stimulated Lymphocytes. The kinetics of the changes in J chain and μ chain RNA expression were determined by analyzing normal murine lymphocytes stimulated with LPS. The initial population of unstimulated cells was isolated from BALB/c splenocytes by 1 *g* velocity sedimentation (25). This method had the advantage that large numbers of unstimulated cells could be

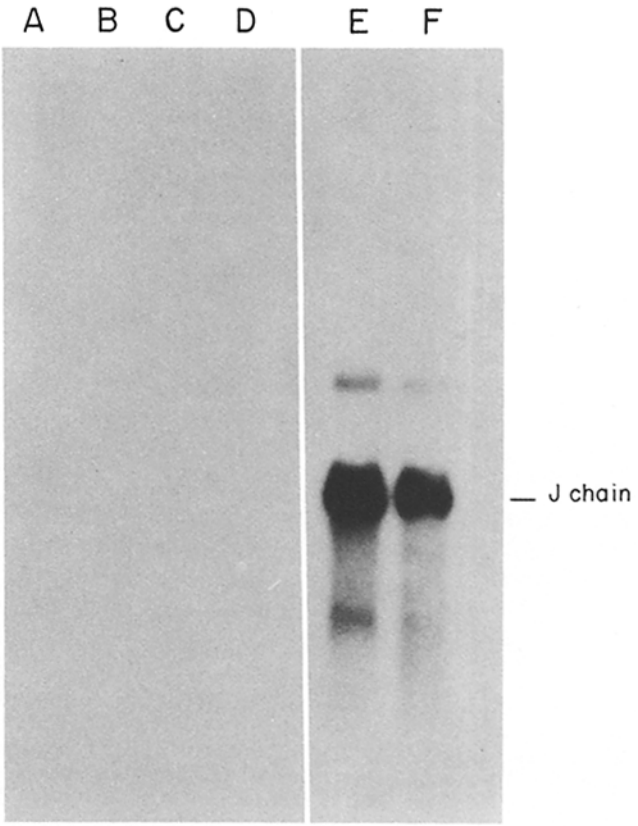

FIGURE 1. Northern blot analysis of J chain–specific mRNA in mouse lymphoid cell lines. ~10 μg of cytoplasmic poly(A)+ RNA from the fibroblast line, L929, and from the lymphocytes 70Z/3, WEHI 231E1, and K46R (*A–D*, respectively) and 0.5 μg of cytoplasmic poly(A)+ RNA from the Ig-secreting lines, M × W 231.1a.2 and MOPC 315 (*E* and *F*, respectively) were size-fractionated by agarose-methylmercury hydroxide electrophoresis, transferred to nitrocellulose, and hybridized to a ^{32}P-labeled J chain cDNA probe.

obtained. By applying 1×10^9 splenocytes to the gradient, $1.1–3 \times 10^8$ small cells were recovered in the fractions taken for analysis. Moreover, the method proved to be highly effective for separation of stimulated and unstimulated B lymphocytes. The recovered cells were invariably of small size and <1 in 2,000 secreted pentamer IgM as determined by reverse plaque assay.

The cells obtained by velocity sedimentation were cultured at densities of 4×10^6/ml in the presence of 20 μg/ml of LPS, and the response was monitored by measurements of cell surface IgM, cell size, and IgM secretion. Fluorescent staining for membrane IgM showed that the initial population contained 25–30% B cells, a value in good agreement with other estimates of the small B cell population in BALB/c mouse spleens (35, 36). After exposure to LPS, the number of cells expressing membrane IgM progressively decreased, and at 120 h constituted ~10% of the population. This change was accompanied by the appearance of blast cells. Although all cells increased slightly in size during the first 24 h of stimulation, a few very large cells were discernible by 48 h. The

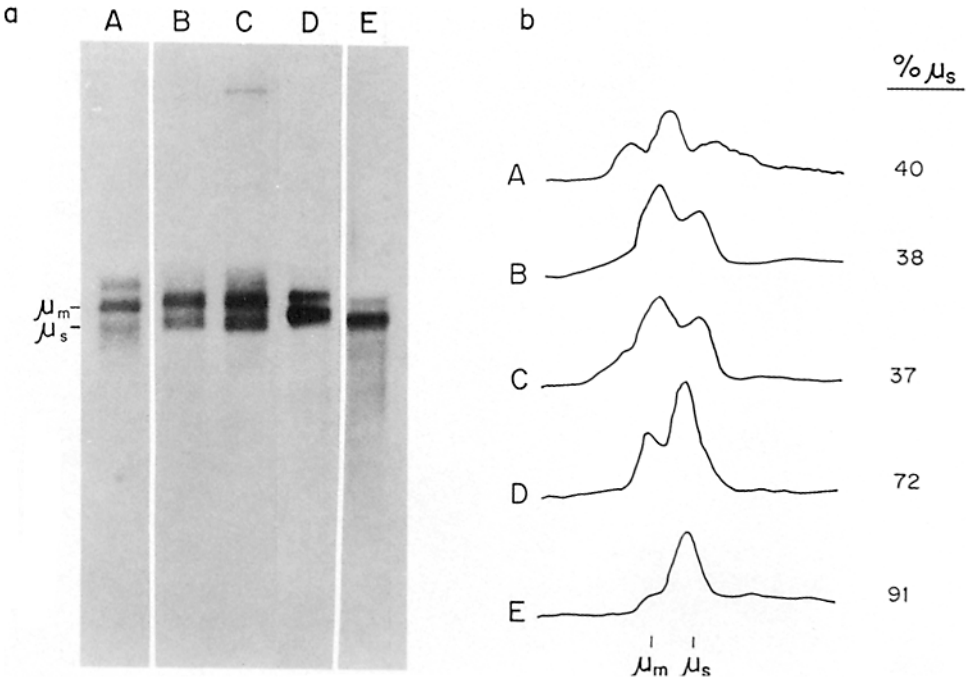

FIGURE 2. Northern blot analysis of μ chain–specific mRNA in mouse lymphoid cell lines. (*a*) ~2.5 μg of cytoplasmic poly(A)+ RNA from the B lymphomas 70Z/3, WEHI 231E1, K46R, and from the IgM-secreting lines, M × W 231.1a.2 and MOPC 104E (lanes *A–E*, respectively) were size-fractionated on 1.4% agarose formamide-formaldehyde gels, transferred to nitrocellulose, and hybridized to a ^{32}P-labeled 10.5 kb *Eco*RI fragment of the constant region of the μ chain gene. (*b*) Densitometer tracings of the respective Northern lanes and the relative percentage of μ_s mRNA.

number of such blasts increased steadily and accounted for 50% of the cell population at 120 h. Ig-secreting cells developed in a different pattern. After 72 h of culture only background levels of plaque-forming cells (PFC) were observed, i.e., 200–400 PFC/10^6 cells. Within the following 24 h, the number of Ig-secreting cells rose by several orders of magnitude and remained at levels of 3–4 × 10^4 per 10^6 cells for another 24 h.

None of the changes induced by mitogen stimulation, loss of membrane IgM, blast formation, or IgM secretion, was detected in small cells cultured in the absence of LPS. The viability of these cells also fell sharply after 48 h of culture, whereas the viability of cells grown in the presence of LPS remained at >85% for at least 96 h.

Analyses of J Chain– and μ Chain–specific RNA in Mitogen-stimulated Lymphocytes. Poly(A)+ RNA was extracted from the initial population of small lymphocytes and from the cultured cells at varying intervals after stimulation. The total number of cells used in each preparation and the yields of poly(A)+ RNA are listed in Table I. Approximately 1-μg samples of the RNA obtained were then assayed for J chain sequences by the Northern blotting procedure. The autoradiographs in Fig. 3 *a* were derived from two separate experiments, one analyzing the 0 h RNA and the other analyzing the RNA from later time points. Every

TABLE I

Analyses of J Chain– and μ Chain–specific RNA in Mitogen-stimulated Small Splenocytes

Time	Number of cells per preparation*	Yield of RNA (μg polyA+)	Relative content per cell	
			J chain RNA	μ chain RNA
h				
0	1.0×10^9 (4)	1.75	1	1
48	2.6×10^8 (1)	0.96	ND‡	5.0
72	3.3×10^8 (1)	2.86	2.1	10.3
84	2.8×10^8 (1)	17.4	1,100	872

* The value in parenthesis indicates the number of sedimentation runs from which the cells were obtained.

‡ No RNA detected.

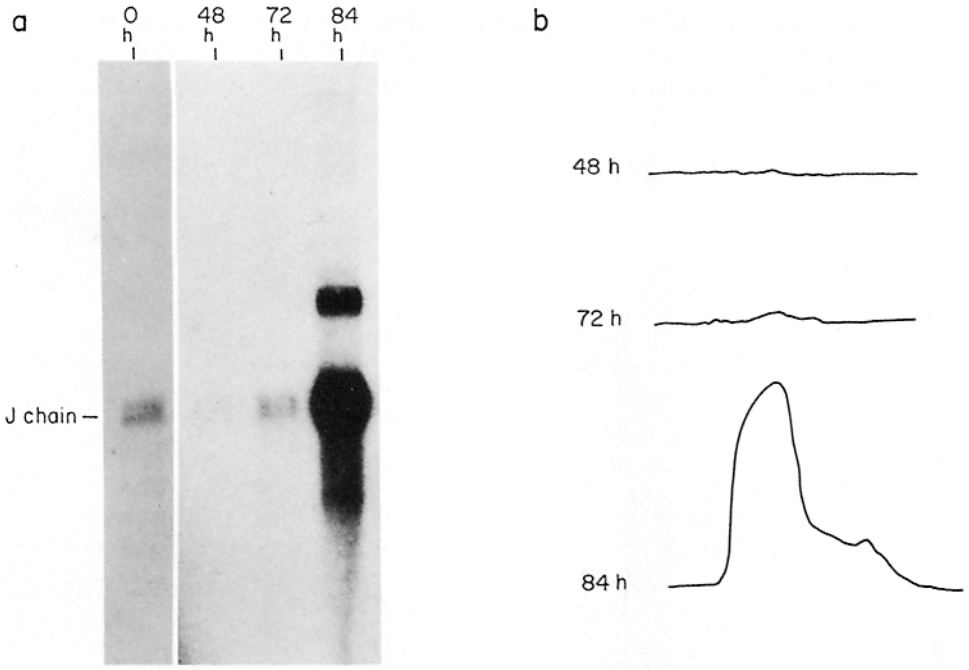

FIGURE 3. Northern blot analysis of J chain–specific RNA in LPS-stimulated mouse splenocytes. (*a*) ~1 μg of total poly(A)+ RNA from the 0, 48, and 72 h cells and 0.5 μg from the 84-h cells were size-fractionated on 1.4% agarose formamide-formaldehyde gels, transferred to nitrocellulose, and hybridized to a ^{32}P-labeled J chain cDNA. (*b*) Densitometer tracings of the 1.5 kb J chain mRNA peak.

effort was made in these experiments to use comparable conditions of transfer, hybridization, and autoradiography. Moreover, equal amounts of the 84-h RNA preparation were run on each gel to serve as standards in the quantitation of the densitometer scans (Fig. 3 *b*). The extent of hybridization was determined from the area under the curve, normalized to the 84-h value on the same gel, and expressed as the amount per cell relative to the amount per cell present in the 0-h sample (Table I).

The data in Fig. 3 and Table I show that the initial population of unstimulated lymphocytes contained a low level of J chain–specific RNA. These small amounts, which were probably contributed by the few secreting cells in the preparation, were no longer detectable in the 48-h RNA, suggesting that the IgM-secreting cells initially present did not survive in culture. The alternative explanation for the absence of J chain RNA, namely that the 48-h sample had a low RNA content, was excluded by the analyses for the same blot with a μ chain probe (Fig. 4). Newly synthesized J chain RNA appeared after 72 h of LPS stimulation, and within just 12 h the amounts present were amplified by several orders of magnitude.

The immobilized RNA used for the J chain analyses was also examined for μ chain–specific sequences (Fig. 4 *a* and *b*). Only the membrane form of μ chain RNA was detected in the 0 h preparation. No band corresponding to a 2.4 kb μ_s species was observed even after longer exposure of the blot (data not shown). There was, however, some background hybridization in the region below the μ_m band that may have obscured the presence of a small amount of μ_s RNA. In contrast to the predominance of μ_m RNA in the 0 h cells, the form of μ chain

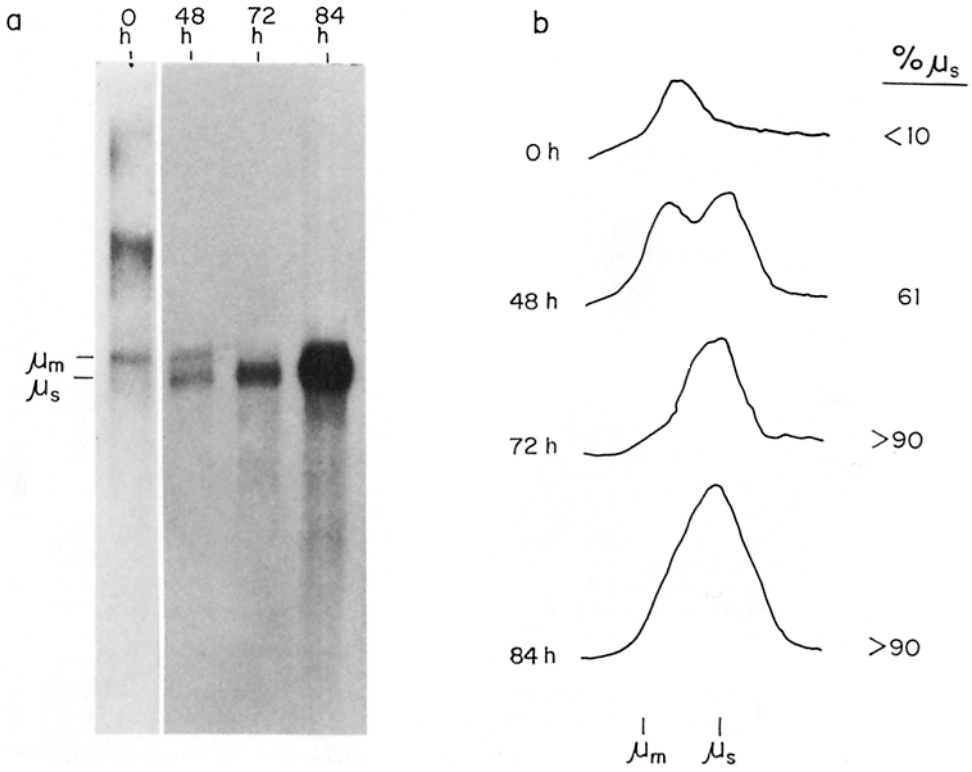

FIGURE 4. Northern blot analysis of μ chain–specific RNA in LPS-stimulated mouse splenocytes. (*a*) The filter shown in Fig. 5 was washed and rehybridized to a ^{32}P labeled 1.3 kb *Hind*III genomic fragment of the μ gene that included the coding region for the Cμ3 and Cμ4 exons. (*b*) Densitometer tracings of the respective Northern lanes and the relative percentages of μ_s mRNA.

RNA expressed shifted dramatically as a function of time after LPS stimulation. At 48 h the secreted form comprised 61% of the μ chain–specific sequences and at 72 h it increased to >90%. These kinetics indicate that the shift to μ_s RNA synthesis begins shortly after stimulation and precedes the appearance of newly transcribed J chain RNA.

Despite the early shift to the μ_s form, the amplification in μ chain RNA content followed a pattern very similar to that observed for J chain RNA. Quantitation of the densitometer scans (Fig. 4 *b* and Table I) showed that the level of μ chain RNA rose very gradually during the first 72 h of stimulation and then increased rapidly in the succeeding 12 h to attain an overall amplification of >800-fold. Thus, in the differentiation of a B lymphocyte to an IgM-secreting cell there must be not only mechanisms for shifting the form of expressed μ chain and for activating J chain gene transcription, but also a mechanism for amplifying the intracellular levels of both μ_s and J chain RNA

Analyses of J Chain and IgM Protein in Mitogen-stimulated Lymphocytes. The relationship between the expression of J chain and μ chain RNA and the expression of their protein products was examined by following the levels of J chain and monomer IgM in the LPS-stimulated lymphocytes. Cells were harvested at time 0 and at 24-h intervals thereafter, and their Ig contents were determined by radioimmunoassay. In addition, aliquots were removed from the culture supernatants and analyzed for the presence of secreted pentamer IgM. The results of such experiments are illustrated in Fig. 5 and summarized in Table II.

The changes in J chain content were found to parallel those observed in J chain mRNA (cf., Fig. 3 and Table II). Very small amounts of J chain, 60 pg/ml of culture, were detected in the unstimulated population, consistent with the presence of low levels of J chain RNA. After LPS activation, the content of J protein, like that of J chain RNA, first dropped below the limit of detection of

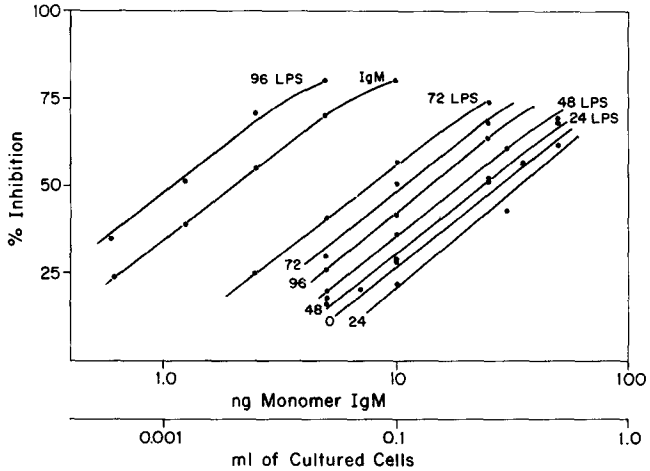

FIGURE 5. Analysis by radioimmunoassay of monomer IgM levels in LPS-stimulated mouse splenocytes. Small splenocytes cultured with and without LPS were lysed at 24-h intervals and the monomer IgM contents of the lysates were determined by comparing the amount required for 50% inhibition with the 50% endpoint of a purified monomer IgM standard.

TABLE II

Levels of J Chain, Monomer IgM, and Pentamer IgM Produced by LPS-stimulated Lymphocytes

Protein	LPS	Sample (hours of culture)					
		0	24	48	72	96	120
Intracellular J chain							
ng/ml cultured cells	+	0.06	ND*	ND	0.26	7.1	24
Relative increase		1.0			4.3	118	400
Intracellular monomer IgM							
ng/ml cultured cells	+		8.0	10	26	182	1,200
ng/ml cultured cells	−	7.5	6.0	8.0	19	14	—
(LPS − no LPS)			2.0	2.0	7	168	1,200
Relative increase			1.0	1.0	3.5	84	600
Secreted pentamer IgM							
ng/ml supernatant	+		0.9	ND	85	1,100	7,300

* Not detected: <200 molecules per cell.

the radioimmunoassay and then increased by several orders of magnitude. A similar correspondence was observed between the changes in monomer IgM and μ chain RNA contents (cf., Fig. 4 and Table II). The unstimulated population contained an average of 23,000 molecules per B cell, a value consistent with the substantial levels of μ_m RNA found in these cells. During the first few days of culture the IgM content rose gradually, paralleling the increase in μ chain RNA, and then amplified >100-fold.

Although the amplifications in RNA and protein contents corresponded in time, they did not in magnitude. The RNA levels increased by a factor of 1,000, whereas the protein levels increased by factors of 10 to 100 over an equivalent period of time. This difference could be accounted for by the assembly of the J chain and monomer IgM into pentameric form and their secretion out of the cell. Radioimmunoassays of the culture supernatants (Table II) showed that pentamer IgM secretion was initiated at 72 h concomitant with the expression of the J chain. The output then rose dramatically as the levels of J and μ chain RNA and the synthesis of their protein products was amplified.

The assays of monomer IgM content did not provide an accurate measure of the IgM synthesized for export. Because only a fraction of the B cell population responded to LPS, the measurements included the membrane form expressed by the unactivated cells as well as the secreted form synthesized by the LPS-responsive cells. To obtain a better estimate of the changes in secreted μ RNA, the IgM content of cells grown in the absence of mitogen was subtracted from the total IgM content of the LPS-stimulated population for each of the time points analyzed (Table II). The corrected values are plotted in Fig. 6 along with the results of the J chain radioimmunoassays. In each case, the contents are expressed as molecules per milliliter of cultured cells. It can be seen that the response curves for J chain and the secreted form of IgM show similar slopes, indicating that the ratio of intracellular J chain to monomer IgM remained constant throughout the amplification phase of the response (37). This relation-

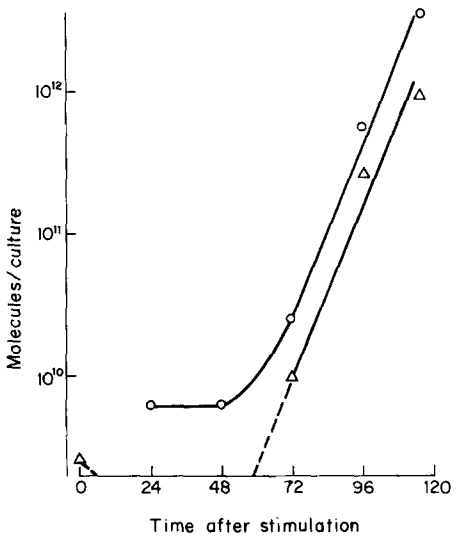

FIGURE 6. Intracellular levels of J chain and monomer IgM in mouse splenocytes stimulated with LPS. Molecules per milliliter of cultured cells of J chain (△) and monomer IgM (○) were calculated from radioimmunoassays. The levels of IgM were corrected for membrane contribution by subtracting the amounts of IgM measured in cells cultured without LPS (see Table II).

ship implies that the synthesis of J chain and monomer IgM, and thus the abundance of their respective mRNAs, is coordinately controlled.

Discussion

By following the expression of μ and J chain RNA in mitogen-stimulated lymphocytes, it was possible to define a time course of differentiative events in the pentamer IgM response. First, synthesis of μ chain RNA shifts from the membrane to the secreted form. Next, synthesis of J chain RNA is initiated. Finally, there is a dramatic amplification in the levels of μ_s and J chain messenger RNA as the B lymphocyte develops into an actively secreting blast cell.

The shift to μ_s RNA synthesis was found to begin shortly after mitogen exposure and within 48 h the secreted form became the predominant species. These data indicate that the change is initiated before the B cells undergo significant proliferation. Studies of the proliferative response have shown that small resting (G_0) B cells do not enter S phase until ~36 h after stimulation and thus do not complete the first round of replication until 6 h later (19). In view of these kinetics, it is likely that the μ_m to μ_s shift is independent of new DNA synthesis. This deduction is consistent with the mechanism of the shift; the form of μ chain RNA expressed is known to be controlled at the level of the primary RNA transcript, either by differential termination of transcription (38) or by differential 3′ cleavage and polyadenylation (4).

In cell lines representing successive stages in B cell differentiation the shift is much less dramatic because significant amounts of μ_s RNA are expressed by the lymphoma counterparts of unstimulated B cells. The secreted form was found to constitute 40% of the μ chain RNA in these lines in contrast to the few percent

expressed by the unstimulated population of small lymphocytes. Thus, the μ_s RNA phenotype of B lymphomas resembles that of normal cells after several days of LPS stimulation rather than that of small resting B cells. These findings suggest that the synthesis of μ_s RNA has been activated in the lymphoma lines during the course of their transformation or during their maintenance in culture.

The expression of J chain RNA was the next event identified in the differentiative process. Newly transcribed J chain sequences first appeared after 72 h of stimulation, indicating that synthesis is initiated between the 2nd and 3rd d of culture, well after the activation of μ_s RNA synthesis and just before the secretion of pentamer IgM. These kinetics are supported by the RNA patterns seen in lymphoid cell lines. No J chain RNA was detected in B lymphoma lines that are comparable to 48-h-activated lymphocytes in their partial shift to μ_s RNA production. On the other hand, large amounts of J chain RNA were found in plasmacytomas representative of the actively secreting cells that develop after 72 h of stimulation. Thus, the kinetics of J chain RNA expression strengthens previous evidence that J chain synthesis is activated at the level of transcription (9) and that the protein product is required for the assembly and secretion of pentamer IgM (21, 37).

The later appearance of J chain RNA may reflect the need for cell division. Once LPS-stimulated lymphocytes complete a first round of replication, they enter a phase of more rapid proliferation, in which they cycle every 16–20 h (39). It is likely, therefore, that the differentiating cells divide twice and possibly three times before J chain RNA is expressed. Cell division could play any of several roles in the initiation of J chain gene transcription: it may be required for the expression of membrane receptors that transmit the initiating signal; alternatively, division may be required to effect the modifications in the J chain gene that allow its expression.

The third change associated with the pentamer IgM response is the rapid amplification in mRNA levels that immediately follows expression of J chain RNA. It was calculated from the relative intensities of hybridization that the content of μ_s and J chain RNA per cell increased by two orders of magnitude between 72 and 84 h after LPS stimulation. Moreover, analyses of protein products indicate that the increases in mRNA abundance are coordinately controlled. Whether the amplification reflects an acceleration in transcription rate, as recent studies of μ chain RNA synthesis in cell lines suggest (40), or whether other mechanisms, such as stabilization of cytoplasmic RNA, may play a role, remains to be determined.

The time course of intracellular changes in LPS-stimulated lymphocytes correlates with the time course of signaling events in the pentamer IgM response. As recent studies have shown, the activation of a B lymphocyte to a secreting cell is dependent not only on antigen binding, but also on the subsequent action of a number of lymphokines (10–18). Cross-linking of surface Ig receptors with an antigen surrogate, anti-μ chain antibody, has been shown to drive resting B cells into the G_1 phase of the cell cycle and make the cells receptive to stimulation by a series of T cell and macrophage products (19). The products identified as necessary for inducing IgM secretion include growth-promoting factors that act early in the differentiative pathway (18, 39) and two classes of T cell–replacing

factors (TRF) (41), one class required within the first 48 h of stimulation and a second that functions as late as 72 h.

The correlation in timing of the membrane and intracellular events suggests the possibility that the successive differentiative changes are triggered by successive lymphokine signals. From the patterns observed it seems likely that the shift to μ_s RNA production is initiated by one of the early-acting stimuli, most likely a TRF, whereas the activation of J chain gene transcription and the amplification in message levels are induced by different late-acting TRFs. However, the possibility remains that the sequence of differentiative events may reflect temporal differences in the mechanisms controlling the μ_s shift, J chain RNA expression, and message accumulation rather than differences in signaling requirements. Once purified lymphokines become available, it should be possible to define their actions by RNA analyses and thus resolve the mechanisms for signaling the pentamer IgM response.

Summary

The time course of differentiative events in the pentamer IgM response was examined by following the expression of J chain and μ chain RNA and their protein products in mitogen-stimulated lymphocytes. The analyses showed that the shift to μ_s RNA synthesis begins shortly after stimulation and precedes proliferation of the cells and any increase in μ RNA levels. In contrast, expression of J chain RNA and the amplification of J chain and μ_s message are late events that coincide with a phase of rapid proliferation and with the secretion of pentamer IgM antibody. The kinetics of J and μ chain RNA expression observed in normal lymphocytes were supported by analyses of lymphoid cell lines. B lymphomas were found to display the RNA pattern characteristic of early-activated lymphocytes, i.e., a partial shift to μ_s RNA production and no J chain RNA, whereas IgM-secreting lines resembled late-activated lymphocytes in their expression of high levels of both μ_s and J chain mRNA. Moreover, the kinetics of J and μ chain RNA expression correlates with the sequential action of B cell lymphokines in the induction of the pentamer IgM response. This correlation suggests that the successive differentiative changes are triggered by successive membrane stimuli.

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