Ig RNA EXPRESSION IN NORMAL B CELLS STIMULATED WITH ANTI-IgM ANTIBODY AND T CELL-DERIVED GROWTH AND DIFFERENTIATION FACTORS

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B lymphocytes use immunoglobulins (Ig) as both membrane receptors for antigens and as secretory products. The membrane-associated and secretory forms of μ heavy (H)[1] chains are specified by mRNAs that are transcribed from a single gene that contains alternative secretory and membrane exons at its 3' end (1–6). Studies using tumor cells of B cell lineage have shown a correlation between the secretory state of the cell and the ratio of mRNA for the membrane form of the μH chain (μ_m mRNA) to mRNA for the secretory form of the μH chain (μ_s mRNA) (2–4, 7, 8). Indeed, in BCL_1 B lymphoma cells, which may be stimulated by a B cell differentiation factor to change from a nonsecretory state to a state of IgM secretion, a change in the ratio of μ_s/μ_m mRNA and an increase in μ_s mRNA occur as the cells initiate IgM secretion (9).

Recently, Lamson and Koshland (10) have shown similar changes in normal B cells stimulated with lipopolysacchride (LPS). Their results indicated that resting B cells expressed principally μ_m mRNA, and that amplification of μ_s mRNA occurs after 72 h in culture, just before the beginning of IgM secretion. The appearance of μ_s mRNA is correlated with the appearance of mRNA for J chain, a molecule key to the formation of pentameric IgM, whose expression is closely associated with IgM secretion (8, 10, 11).

We have recently (12, 13) developed a system in which B cell activation, proliferation, commitment to IgM secretion, and entry into the IgM secretory state are separately controlled events. Normal resting B cells cultured with low concentrations of anti-IgM antibody are activated, and will proliferate, in the presence of B cell stimulatory factor (BSF)-pl (14) (formerly designated B cell

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growth factor; 15). The development of these cells into cells synthesizing IgM at high rates depends upon the action of two additional T cell products (13). One of these, B15-TRF (T cell–replacing factor), which is found in the supernatant of the B151K12 T cell hybridoma (16), is required early in culture and appears to be associated with the commitment of B cells to differentiation. The second factor, EL-TRF, found in supernatants of induced EL-4 cells, is required late in the culture and causes differentiation of appropriately prepared B cells into an IgM-secreting state (13). Recently, we have shown (17) that induced EL-4 supernatants contain two distinct molecules with EL-TRF activity. One is inter-leukin 2 (IL-2); the second is a 32,000 dalton molecule that lacks IL-2 and γ-interferon activity.

This system allows a detailed study of the relationship between each step in the B cell response process and the appearance of mRNA involved in the secretion of IgM. In this paper, we have examined levels of μ_m mRNA, μ_s mRNA, and total μH chain mRNA, as well as J chain mRNA and κ chain mRNA in resting B cells, and of anti-IgM–stimulated B cells that have been cultured with various T cell–derived growth and differentiation factors. Our results indicate that a striking induction of μ_s mRNA and J chain mRNA, and an increase in the μ_s/μ_m mRNA ratio occur at the end of the response process, when all T cell–derived growth and differentiation factors have acted upon these cells. No major specific changes are detected in cells that have completed only a part of the total response.

Materials and Methods

Mice. BALB/c mice were obtained from The Jackson Laboratory, Bar Harbor, ME, and used at 8–12 wk of age.

Reagents. Affinity-purified goat anti-mouse IgM antibody was prepared as previously described (18).

Culture Medium. RPMI 1640 (Biofluids, Rockville, MD) supplemented with 10% fetal calf serum (FCS), penicillin (50 μg/ml), streptomycin (50 μg/ml), gentamycin (100 μg/ml), L-glutamine (2 mM), and 2-mercaptoethanol (2-ME) (5 × 10⁻⁵ M), was used as culture medium in these experiments. In the preparation of EL-4 supernatants, RPMI 1640 medium containing 1% FCS, with supplements described above, was used.

Factor Preparation. EL-4 supernatant was prepared by culturing a cloned subline of EL-4 thymoma cells (2 × 10⁶/ml) with 10 ng/ml of phorbol myristate acetate (PMA) for 48 h (19). Cell-free supernatant was collected and PMA was removed as described (14). Partially purified BSF-p1 and EL-TRF were obtained from this cell-free supernatant by phenyl-Sepharose column chromatography (13, 20). BSF-p1 was also purified by reverse phase high pressure liquid chromatography (HPLC). Its preparation will be described in detail elsewhere.[2] BSF-p1 activity was tested in a B cell costimulator assay (14). B15-TRF is the culture supernatant of B151K12 T hybridoma cells (16). Molecularly cloned human IL-2, purified by HPLC, was kindly provided to us by Dr. J. Farrar, Hoffmann-LaRoche, Inc., Nutley, NJ. IL-2 activity was measured by the stimulation of uptake of [³H]thymidine by an IL-2-dependent cell line (HT-2) (21). One unit of IL-2 is defined as the reciprocal of the dilution required to cause half-maximal proliferation of 4 × 10³ HT-2 cells.

B Cell Preparation. We prepared B cells by pretreating donor mice with anti-thymocyte serum, followed by passing spleen cells over a Sephadex G10 column, and two rounds of complement-mediated lysis with monoclonal antibodies (mAb) anti-Thy-1.2 and anti-Lyt-1.2 (22).

Assay for IgM-synthesizing Cells and J Chain Production in Stimulated B Cells. 2 × 10⁵ purified B cells were cultured in flat-bottom, 96-well microtiter plates (Costar 3596) in

0.2 ml of medium containing 5 μg/ml of goat anti-IgM and various combinations of factors. After 4 d in culture, the presence of cytoplasmic IgM was determined by immunofluorescence as previously described (13). The amounts of cellular J chain were determined on day 4 by radioimmunoassay (23).

Cell Lines. The plasmacytomas MOPC-104E (μ, λ_1) and MOPC-315 J (α, λ_2) (provided by Dr. R. Lynch, University of Iowa College of Medicine, Iowa City, IA), and the lymphoma L10A (μ, λ) (given to us by Dr. R. Asofsky, NIH, Bethesda, MD), were maintained in tissue culture in RPMI 1640 containing 10% FCS, antibiotics, 5 × 10^{-5} M 2-ME, and 2 mM L-glutamine. All cell lines were used for preparation of cytoplasmic RNA. L10A cells were also used to obtain oligo(dT)-selected mRNA.

DNA Probes. The μ_m-specific probe was made by digestion of the genomic DNA clone μM8A with HindIII to yield a 2.5 kilobase (kb) fragment of the μH gene, containing the membrane, but not the secretory portion of the gene (P. Early, unpublished data). We prepared from this a Kpn 1-Pst 1 fragment of 731 base pairs (bp). This fragment lacks several repetitive sequences present in the 2.5 kb fragment (24) and hybridizes only with the membrane exon of the μH gene and portions of the flanking introns; it was designated μ_m (Fig. 1 A).

A secretory type μH gene–specific probe was subcloned from the cDNA clone p104Eμ12. p104Eμ12 was kindly provided by Dr. R. Wall, UCLA School of Medicine, Los Angeles, CA. We first digested p104Eμ12 with Pst 1. This Pst 1 fragment was further digested with Sau 3AI to yield a 191 bp fragment containing the secretory portion and 3' untranslated segment of the secretory form of μ_m mRNA. The resultant Pst 1-Sau 3AI fragment was inserted into the plasmid pUL9. This plasmid specifically hybridizes with mRNA of the secretory form and was designated μ_s. The μ_{CH} (μ heavy chain constant region) cDNA probe, kindly provided by Dr. K. Marcu (State University of New York, Stony Brook, NY), contained the coding regions for CH$_1$ to CH$_4$ (25) (Fig. 1); it was used to detect both types of μ mRNA.

To detect J chain–specific sequences, the cDNA insert from the clone pJc21 was used for hybridization (26). The properties of this clone have been described. The κ light chain probe, kindly provided by Dr. R. Wall, is a recombinant plasmid containing ~600 nucleotides of κ light chain mRNA sequences, including the entire constant region and <100 nucleotides of the κ light chain variable region (27). The cDNA clone pMHC-1, encoding amino acids 66–286 of the H-2L^d molecule, prepared by G. A. Evans et al. (28), was provided by Dr. D. H. Margulies (National Institute of Allergy and Infectious Diseases, NIH), and was used for detection of L^d mRNA expression in B cells. DNA plasmids and fragments to be used for hybridization were [^{32}P]-labeled by nick translation (29).

Isolation of Cytoplasmic RNA. Cultured cells were collected and washed twice with chilled Dulbecco's phosphate-buffered saline (Dulbecco's PBS), and cytoplasmic RNA was prepared as described (30). Briefly, cells were resuspended in ice-cold 0.14 M NaCl/0.01 M Tris-HCl, pH 8.4, and 0.0015 M MgCl$_2$ containing 250 μg of heparin and 250 μg of spermidine per ml, and lysed by the addition of Nonidet P-40 (NP-40) to 0.5%. After the nuclei were removed from the lysate by centrifugation, cytoplasmic RNA was obtained by sequential extraction, twice with phenol, and once in chloroform/isoamyl alcohol (24:1). Cytoplasmic RNA was precipitated from solution in 70% ethanol at -20°C and then dissolved in 10 mM Tris-HCl, pH 7.4 and 1 mM EDTA. Poly(A)$^+$ RNA was prepared from cytoplasmic RNA by using an oligo(dT) cellulose column. Sterile siliconized tubes were used in all steps to avoid nonspecific binding of RNA and to minimize RNA degradation.

Dot Blotting of Cytoplasmic RNA onto Nitrocellulose Filters. Cytoplasmic RNA was denatured by heating at 60°C for 15 min in the presence of 7.4% formaldehyde. Each sample was serially diluted with 15× SSC (1× SSC is 0.15 M NaCl in 0.015 M sodium citrate) and was blotted onto nitrocellulose paper with a suction manifold, fixed by baking, and then used for hybridization.

Northern Blot Analysis of Poly(A)$^+$ RNA and Cytoplasmic RNA. Poly(A)$^+$ RNA and cytoplasmic RNA was denatured by heating at 60°C for 15 min in the presence of 50% (vol/vol) formamide, 20% (vol/vol) formaldehyde, and MOPS buffer (0.02 M morpholi-

A. Genetic Map of μ_{CH}, μ_S, and μ_M Probes
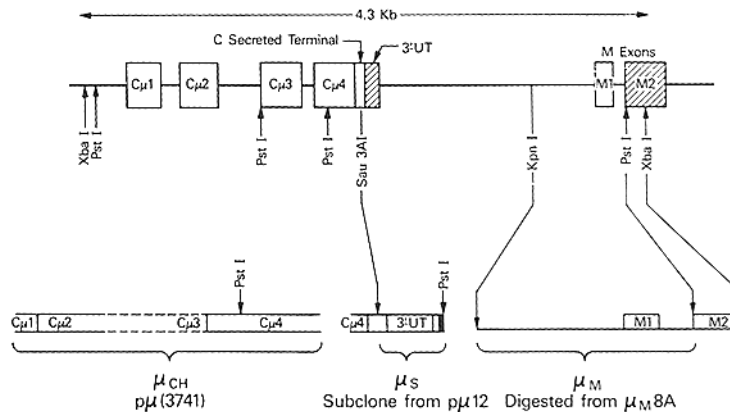
B. μ_M and μ_S Detect mRNAs of Distinct Size
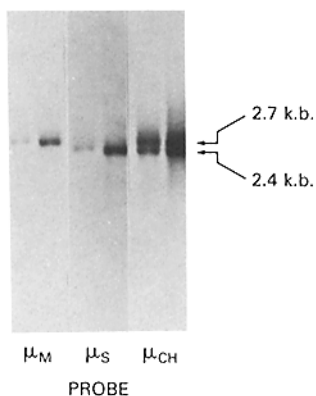
C. Specificity of μ_M and μ_S Probes

Probe	Source of RNA	1/Dilution of RNA							
		1	2	4	8	16	32	64	128
μ_M	MOPC 104E								
	MOPC 315J								
	L10A								
μ_S	MOPC 104E								
	MOPC 315J								
	L10A								

FIGURE 1. (A) Restriction map of the Cμ gene. The upper map illustrates the genomic restriction map of Cμ. Open boxes represent exons; horizontal lines designate introns. Shaded boxes are 3′ untranslated sequences present in either μ_m or μ_s mRNA. The lower portion of the figure shows DNA used as probes for hybridization. μ_{CH} [pU (3741)] is a cDNA probe that commences at the end of the Cμ1 and terminates near the end of Cμ4. μ_s is a 191 bp cDNA clone containing a part of the secretory region, the 3′ untranslated region, and a synthetic DNA composed of a linker and the reconstituted plasmid Pst 1 site. μ_m is a 731 bp Kpn 1–Pst 1 fragment of the genomic DNA clone μM8A. (B) Cytoplasmic polyadenylated RNA from L10A was subjected to electrophoresis through a 0.8% formaldehyde/agarose gel, transferred to nitrocellulose filters, and hybridized with ^{32}P-labeled μ_{CH}, μ_m, or μ_s probes. (C) RNA from MOPC-104E, MOPC-315 J, and L10A was dotted onto nitrocellulose filters. Dots were hybridized with ^{32}P-labeled μ_m and μ_s probes. Autoradiographs were made by exposing x-ray film to these dots. Exposures for μ_{CH} were 20 h in duration; μ_m and μ_s were exposed for 56 h.

nopropanesulfonic acid, 5 mM sodium acetate, 0.5 mM EDTA), followed by cooling on ice. This denatured RNA was mixed with 1/10th volume of loading dye (50% glycerol and 0.5% bromophenol blue), and then applied to a 0.8% agarose horizontal gel containing 1.2 M formaldehyde (31). The sample was electrophoresed at 30–40 V for 16 h, transferred to nitrocellulose paper, and baked 2 h at 80°C under negative pressure.

Hybridization with Probes and Autoradiography.   After incubation of nitrocellulose filters with nick-translated plasmid DNA or DNA fragments for 16 h at 45°C, the filters were rinsed in 2× SSC and 0.1% (wt/vol) sodium dodecyl sulfate (SDS) at room temperature for 30 min. This was followed by two washes in the same buffer for 30 min at 60°C and two washes in the 0.2× SSC and 0.1% (wt/vol) SDS for 30 min at 60°C. Kodak XAR-5 x-ray film was exposed to the filters for 1–3 d at −70°C, using intensifying screens to obtain autoradiographs. The resultant autoradiographs were scanned with a densitometer.

Results

Specificity of μ_s and μ_m Probes.   We wished to verify the specificity of our probes for the membrane and secretory forms of μH mRNA. The μ_s probe is a cDNA subclone, derived in our laboratory from p104Eμ12 (1), and the μ_m probe is a Kpn 1–Pst 1 fragment derived from the genomic clone μM8A (Fig. 1A). When analyzed by Northern blotting, μ_m hybridized with a poly(A)$^+$ RNA of 2.7 kb while μ_s hybridized with 2.4 kb material, consistent with the established sizes for μ_m and μ_s mRNA (1–6). μ_{CH}, a cDNA probe complementary to the CH$_1$ to CH$_4$ domains of the μH chain gene, hybridized with both forms of mRNA (Fig. 1B). Both μ_s and μ_m hybridized extensively with cytoplasmic RNA from MOPC-104E and L10A, both of which express IgM. μ_m failed to hybridize at all with cytoplasmic RNA from MOPC-315 J, an α, λ_2 plasmacytoma, from which the genomic μH chain gene has been deleted, while μ_s hybridized to a minor degree to MOPC-315J RNA (Fig. 1C). This small degree of hybridization was not observed under more stringent washing conditions.

Changes in μ_m and μ_s mRNA Expression in B Cells Stimulated with Anti-IgM and T Cell–derived Factors.   Resting B cells were cultured in the presence of anti-IgM and BSF-p1. B15-TRF and/or EL-TRF were added to some cultures. At 4 d, cells were collected, and cytoplasmic RNA was extracted and dotted onto nitrocellulose paper. In each case, the initial dot contained 10 μg of RNA; subsequent dots contained twofold dilutions of this. The yield of RNA from B cells cultured with anti-IgM and BSF-p1, with or without EL-TRF, was similar. Cultures containing B15-TRF yielded about half as much RNA as those containing anti-IgM and BSF-p1 without B15-TRF. We also dotted 10 μg RNA from L10A cells and from freshly isolated resting B cells. It should be noted that the yield of RNA from resting B cells is much lower (5–10-fold) than that from each of the activated B cell populations.

Fig. 2 presents autoradiographs of the hybridized dots, and Fig. 3 presents quantitative analysis of these autoradiographs using densitometry tracing to evaluate the relative intensity of individual dots hybridized with μ_m or μ_s. B cells stimulated with anti-IgM and BSF-p1 display an ~2.8-fold enhancement in the expression of μ_m mRNA when compared with resting B cells. Since this comparison is based on fixed amounts of cytoplasmic RNA, and the yield of cytoplasmic RNA from resting B cells is $\frac{1}{5}$–$\frac{1}{10}$ that obtained from stimulated B cells, these results indicate that, on a per cell basis, a marked enhancement in expression of μ_m mRNA occurs. When we made direct comparisons, based on amounts of cytoplasmic RNA from the same number of B cells, we found enhancements of 8–16-fold in μ_m mRNA expression in B cells stimulated with anti-IgM and BSF-p1. Adding B15-TRF, EL-TRF, or both caused little or no change in μ_m mRNA
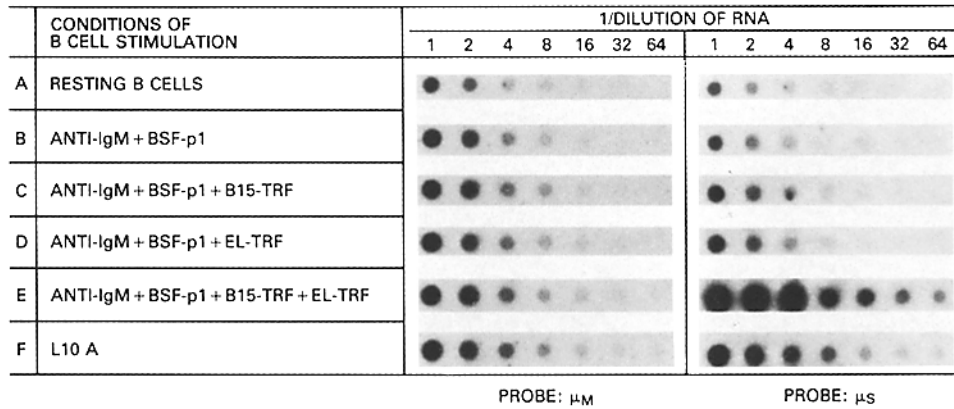
CONDITIONS OF B CELL STIMULATION	1/DILUTION OF RNA													
	1	2	4	8	16	32	64	1	2	4	8	16	32	64
A RESTING B CELLS														
B ANTI-IgM + BSF-p1														
C ANTI-IgM + BSF-p1 + B15-TRF														
D ANTI-IgM + BSF-p1 + EL-TRF														
E ANTI-IgM + BSF-p1 + B15-TRF + EL-TRF														
F L10 A														

PROBE: μ_M PROBE: μ_S

FIGURE 2. Cytoplasmic dot-blot hybridization with μ_m and μ_s. B cells were prepared as described in Materials and Methods. 2×10^5 B cells were cultured in 0.2 ml of medium containing 5 μg/ml of anti-IgM and various combinations of cofactors. Such cells were cultured alone, or with BSF-p1 (reverse phase HPLC-purified material from induced EL-4 supernatant, containing 40 U of BSF-p1/ml), B15-TRF (equivalent to 125% of supernatant from B151K12 hybridoma cells), and EL-TRF (5% of an IL-2-rich phenyl-Sepharose fraction of EL-4 supernatant, containing 80 U of IL-2/ml) either individually or in various combinations. After 4 d of incubation, cultured B cells of each group were pooled and washed twice with cold PBS. Cytoplasmic RNA was prepared from cultured and resting B cells by NP-40 lysis, sequential extraction with phenol and chloroform/isoamylalcohol (24:1), followed by ethanol precipitation. The initial dots contained 10 μg of RNA and subsequent dots contain serial twofold dilutions of this. Two replicas were made from each sample, of which one was hybridized with μ_m and the other with μ_s.

expression, compared with the change in cytoplasmic RNA levels from B cells stimulated with only anti-IgM and BSF-p1.

μ_s mRNA levels, on a constant cytoplasmic RNA basis, are very similar in resting B cells compared with B cells that have been stimulated with anti-IgM or BSF-p1 plus B15- or EL-TRF. Addition of all three factors causes an enhancement of 20–26-fold in μ_s mRNA expression. Indeed, as shown in Table I, this marked enhancement in μ_s expression correlates with the appearance of cells with cytoplasmic IgM. When compared on a per cell basis, B cells stimulated with anti-IgM and all three factors display an increase in μ_s mRNA levels of ~170-fold over resting B cells (Table I). This increase is similar in magnitude to the increase in μ_s mRNA levels observed in LPS-activated cells (10).

The ratio of μ_s/μ_m mRNA in each of these cell populations can be estimated by reference to the μ_s/μ_m mRNA ratios in cytoplasmic RNA of L10A cells. A Northern blot analysis of cytoplasmic RNA from L10A cells, hybridized with μ_{CH}, is presented in Fig. 4. The relative intensity of the μ_s (2.4 kb) and μ_m (2.7 kb) species (determined by densitometry tracing) was 1.22. Since the same preparation of L10A cytoplasmic RNA was included in the dot blotting experiment illustrated in Figs. 2 and 3, we could use the known μ_s/μ_m ratio of L10A RNA, together with the expression of μ_m and of μ_s in each cell population relative to that in L10A cells, to calculate μ_s/μ_m ratios for these RNA. Based on this, we observed μ_s/μ_m ratios of 0.31–0.40 in RNA from B cells treated with anti-IgM, or BSF-p1 alone, or with B15- or EL-TRF. When all three T cell factors are present, a μ_s/μ_m ratio of 5.6 is obtained.
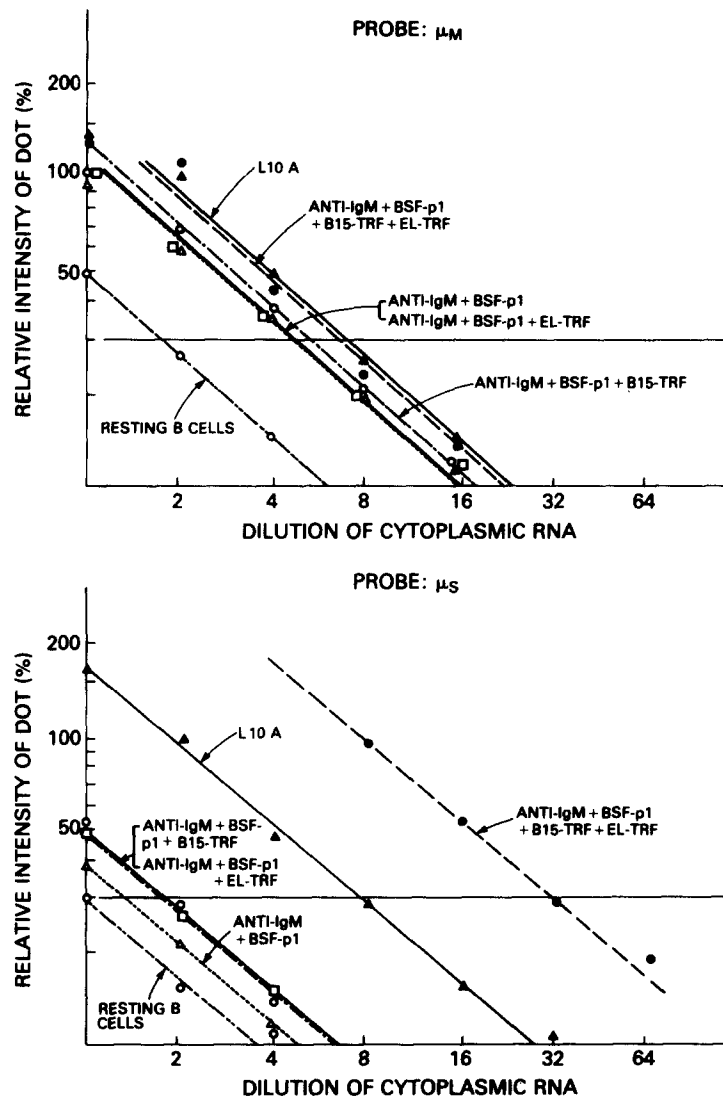
FIGURE 3. Densitometery scanning of RNA dots. Autoradiographs were scanned with a densitometer and recorded, on the ordinate, relative to that of the 5-μg dot from L10A cells, which was assigned a value of 100%. Dilutions yielding 30% relative intensity were used to compare μ$_s$ and μ$_m$ RNA content.

Changes in Expression of J Chain mRNA and Protein in Stimulated B Cells. It has been shown (8, 10, 11, 32, 33) that IgM secretion, in both transformed B cell lines and LPS-stimulated normal B cells, is dependent upon expression of J chain mRNA and protein. To allow a more precise examination of the relationship between J chain synthesis and IgM secretion, we examined the control of J chain expression in B cells stimulated with anti-IgM and the T cell–derived growth and differentiation factors. In these experiments, we compared mRNA expression, based on a constant number of B cells. Resting B cells have a barely

TABLE I

Change of Ratio of μ_s and μ_m mRNA in Anti-IgM–stimulated B Cells

Conditions of B cell stimulation	Percent cyto-plasmic IgM+ cells	Relative content (%)		μ_s/μ_m Ratio
		μ_m	μ_s	
Resting B cells	NT	24	13	0.66
Anti-IgM + BSF-p1	0.5	67	17	0.31
Anti-IgM + BSF-p1 + B15-TRF	0.7	75	22	0.36
Anti-IgM + BSF-p1 + EL-TRF	0.4	67	22	0.40
Anti-IgM + BSF-p1 + B15-TRF + EL-TRF	20.7	96	440	5.59
L10A	NT	100	100	1.22

Relative content of RNA hybridizable with μ_m and μ_s was determined by densitometry as shown in Fig. 3. μ_s/μ_m ratios were calculated based on a μ_s/μ_m ratio of 1.22 for L10A cytoplasmic RNA, shown in Fig. 4. NT, not tested. In these experiments, similar amounts of cytoplasmic RNA were "dotted" in each group. The yield of RNA per resting B cell was 5–10-fold less than that of stimulated cells. Thus, the increase in μ_m and μ_s RNA between resting and activated B cells, on a per cell basis, is 5–10-fold greater than that calculated from relative μ_m and μ_s content.
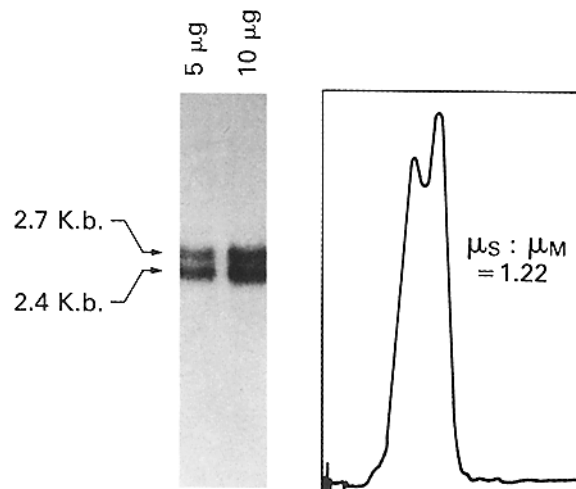

FIGURE 4. Northern blot analysis of L10A cytoplasmic RNA. Cytoplasmic RNA (5 and 10 μg) from L10A was electrophoresed through a 0.8% formaldehyde/agarose gel. After transfer to a nitrocellulose filter, the RNA was hybridized with ^{32}P-labeled μ_{CH} probe. The sizes of the two μ mRNAs were deduced from calibration with ribosomal RNA markers visualized by ethidium bromide staining. The autoradiograph was scanned and the relative intensities of these two bands were determined to be 1.22.

detectable level of J chain mRNA (Fig. 5). Stimulation with anti-IgM and BSF-p1 causes a 2–4-fold enhancement in this level; addition of EL-TRF causes no further enhancement in J chain mRNA, while addition of B15-TRF causes a slight increase. However, if both B15- and EL-TRF are added to B cells already stimulated with anti-IgM and BSF-p1, a further 4–8-fold enhancement in J chain
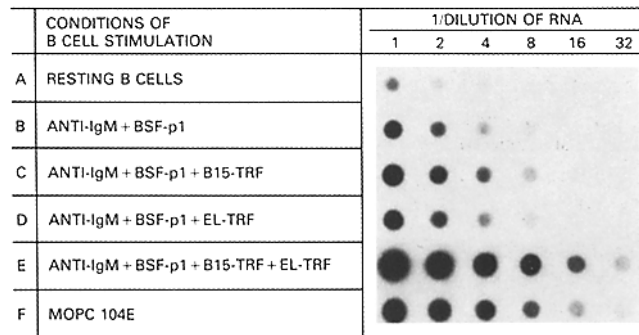
| CONDITIONS OF | 1/DILUTION OF RNA | | | | | |
B CELL STIMULATION	1	2	4	8	16	32
A RESTING B CELLS						
B ANTI-IgM + BSF-p1						
C ANTI-IgM + BSF-p1 + B15-TRF						
D ANTI-IgM + BSF-p1 + EL-TRF						
E ANTI-IgM + BSF-p1 + B15-TRF + EL-TRF						
F MOPC 104E						

FIGURE 5.   Expression of J chain mRNA in stimulated B cells. 2×10^5 B cells were cultured with anti-IgM and various combinations of T cell-derived factors for 4 d. Cytoplasmic RNA was prepared from 10^7 B cells by NP-40 lysis followed by phenol and chloroform/isoamylalcohol (24:1) extraction. In each case, the initial dot contained cytoplasmic RNA obtained from 1.25×10^6 B cells. Subsequent dots contained serial twofold dilutions of this. Five replicas were made and were hybridized with five different probes (J chain probe in Fig. 5; κ chain probe in Fig. 7; L^d probe in Fig. 8; μ_s and μ_{CH} were also used but data are not shown). Simultaneously, each culture was examined for percentage of cells with cytoplasmic IgM and for J chain protein content (Fig. 6B). The BSF-p1 used in this experiment was a phenyl-Sepharose fraction of EL-4 supernatant.

mRNA is observed. To examine the correlation between J chain mRNA expression and μ_s mRNA expression, we compared replica dots that were hybridized with either μ_s or the J chain probe. The result showed a strong correlation between the expression of these two genes (data not shown).

The enhancement in J chain mRNA synthesis correlates very well with the presence of J chain protein in stimulated B cells. There was insufficient J chain to precisely quantitate (Fig. 6), except in cells grown in the presence of all three stimulatory factors, where there was a substantial enhancement in the expression of J chain. Indeed, these stimulated B cells had a J chain content similar to that of the BALB/c plasmacytoma MOPC-104E. The induction of J chain expression correlated very well not only with the expression of μ_s mRNA but also with the appearance of cells with cytoplasmic IgM.

Changes in Expression of κ Chain mRNA and mRNA for Class I Major Histocompatibility Complex (MHC) Antigens.   To further examine the induction of Ig mRNA in the course of B cell responses, we probed dot blots with a κ-specific cDNA probe. This experiment used replica blots of those probed with J chain cDNA in Fig. 5; a constant number of cells was compared. We observed a low level of κ mRNA in resting B cells and a 4–8-fold induction upon stimulation with anti-IgM and BSF-p1, with no further changes when B15- or EL-TRF was added (Fig. 7). In the presence of all three factors, there was a substantial increase (about fourfold), to levels comparable to that of MOPC-104E cells. In general, changes in expression of κ mRNA closely paralleled changes in expression of total μ mRNA, as detected with μ_{CH} (data not shown).

Additional replicas of these dots were hybridized with a cDNA specific for the class I MHC gene L^d, in order to provide information on changes in expression of mRNA for a molecule not known to be involved in the process of B cell proliferation or differentiation. As shown in Fig. 8, B cells stimulated with anti-
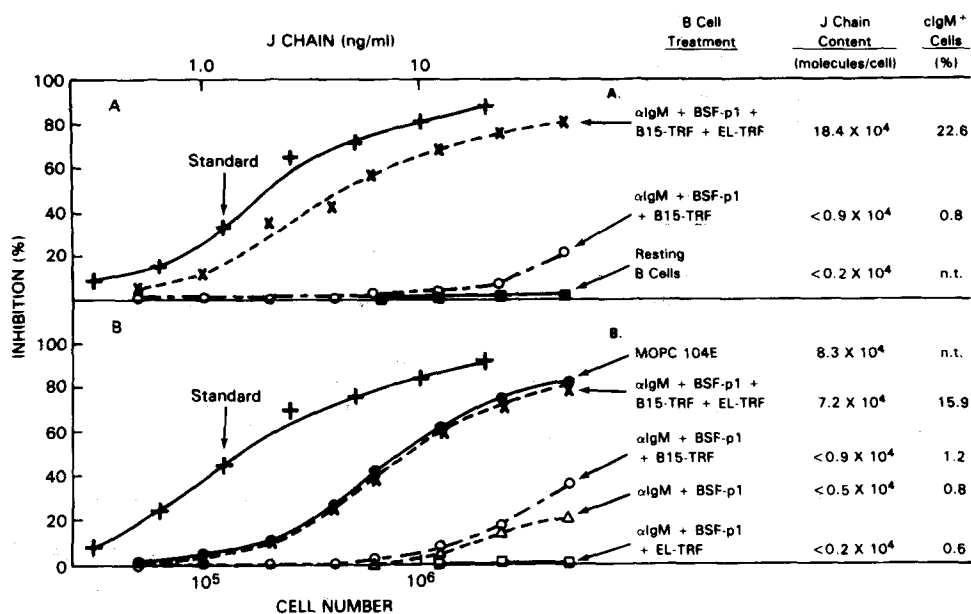
FIGURE 6. Analysis of J chain protein production by B cells stimulated with anti-IgM and T cell–derived factors. B cells were cultured with anti-IgM and T cell–derived factors. Cell lysates were made by NP-40 (1%) lysis. Such lysates were examined for their capacity to inhibit the binding of ^{125}I–J chain to goat anti-J chain antibodies. The abscissa represents the amounts of J chain (for standard preparations) or number of cell equivalents (for cell lysates); the ordinate represents the percent inhibition. A and B are separate experiments. In B, the B cells used are the same as used for analysis of J chain mRNA expression shown in Fig. 5.

FIGURE 7. Expression of κ chain mRNA in stimulated B cells. One of the replicas described in Fig. 5 was hybridized with the κ chain probe.

IgM and BSF-p1 display an eightfold increase in levels of L^d mRNA compared with resting B cells. This suggests that changes in levels of μ, J chain, and κ light chain mRNA found in activated compared with resting B cells may not have significance specific to the activation process. Levels of L^d mRNA do not change upon further addition of T cell factors, including situations in which all three factors are present. This indicates that the increase of μ_s, J, and κ mRNA, and

| CONDITIONS OF | 1/DILUTION OF RNA | | | | | |
B CELL STIMULATION	1	2	4	8	16	32
A	RESTING B CELLS						
B	ANTI-IgM + BSF-p1						
C	ANTI-IgM + BSF-p1 + B15-TRF						
D	ANTI-IgM + BSF-p1 + EL-TRF						
E	ANTI-IgM + BSF-p1 + B15-TRF + EL-TRF						
F	MOPC 104E						

FIGURE 8.   Expression of L^d mRNA in stimulated B cells. A replica of the filters used in Fig. 5 and Fig. 7 was hybridized with a cDNA probe complementary to L^d molecule.

the changes in the μ_s/μ_m ratios in such cases, are specifically related to the differentiation process.

Time Course of Induction of μ_s and J Chain mRNA.   To further examine the relation between synthesis of μ_s and J chain mRNA, and between the expression of these mRNAs and the induction of high rate IgM synthesis, we examined the time course of expression of μ_s and J chain mRNA and the appearance of cytoplasmic IgM^+ cells among cells that had been stimulated with anti-IgM, BSF-p1, B15- and EL-TRF (Fig. 9). In these experiments, we used molecularly cloned human IL-2 as a source of EL-TRF (17). Levels of μ_s mRNA that exceeded those found in B cells cultured with anti-IgM and BSF-p1 (with or without B15-TRF), were first detected at 72 h of culture with anti-IgM and all three factors. At that time, the percentage of cells with cytoplasmic IgM was only slightly greater than in control populations. By 96 h, a striking increase in μ_s mRNA occurred, depending on the concentration of IL-2 present. A striking increase in the percentage of cells with cytoplasmic IgM was also observed. Changes in levels of J chain mRNA were closely correlated to those of μ_s mRNA, in terms of the time after initiation of culture and in the concentrations of IL-2 required for the observed increases (Fig. 9). These results strongly reinforce the concept of an intimate relationship between μ_s and J chain mRNA expression and IgM secretion.

Discussion

Several investigators (1–8) have found a correlation between the IgM secretory state in tumors of the B cell lineage and high levels of mRNA for μ_s, as well as high μ_s/μ_m mRNA ratios. Furthermore, IgM synthesis, induced by LPS (10) in normal B cells, and by B cell differentiation factor ($BCDF_\mu$) in the B lymphoma BCL-1 (9), is associated with striking increases in μ_s mRNA and with changes in μ_s/μ_m ratios. Our current experiments examine the relationship between changes in μ_s, μ_m, and related mRNA, and a high IgM synthesis rate in a system where it is possible to bring normal cells to various stages along the B cell response pathway by controlling which growth and differentiation factors are provided in tissue culture.
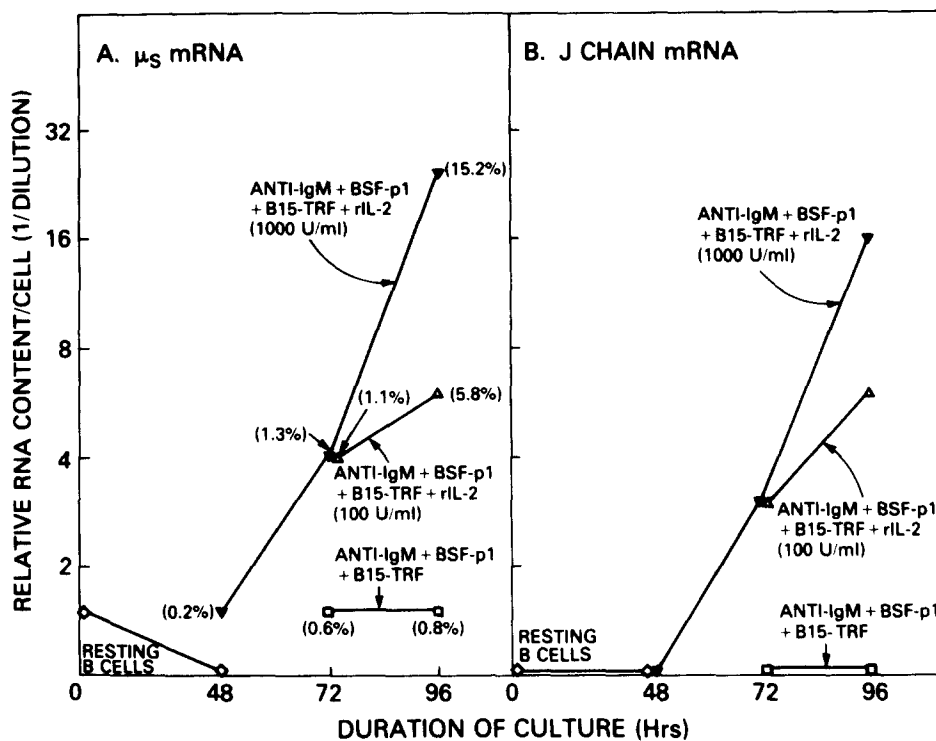
FIGURE 9. Time course of induction of μ_s and J chain–specific mRNA. B cells stimulated with anti-IgM and T cell–derived factors were harvested at 48, 72, and 96 h after the initiation of culture. In this experiment, BSF-p1 is a reverse phase HPLC-purified preparation; molecularly cloned IL-2 was used as EL-TRF. These cell populations were used for preparation of cytoplasmic RNA, and were also examined for the presence of B cells with cytoplasmic IgM. Numbers in parentheses are the percent of cytoplasmic IgM$^+$ cells in each group. As a negative control for activated B cells, cytoplasmic RNA was prepared from B cells cultured in medium containing no anti-IgM or T cell–derived factors, for 48 h. Each preparation of RNA was dotted onto a nitrocellulose filter and probed with either the μ_s or J chain probe. The initial dot contained the amount of cytoplasmic RNA obtained from 10^6 cells; subsequent dots contained twofold dilutions of this. Although not shown, a group of cells was stimulated with anti-IgM and BSF-p-1. These cells had levels of μ_s and J chain mRNA and of cytoplasmic IgM$^+$ cells similar to that of cells stimulated with anti-IgM, BSF-p-1, and B15-TRF.

Our results indicate that striking changes occur in the level of μ_s and J chain mRNA in B cells stimulated with anti-IgM, BSF-p1, B15-TRF, and EL-TRF. As we have already shown, induction of high IgM synthesis rates in B cells cultured with anti-IgM depends upon the presence of each of these T cell–derived factors. Thus, the striking increases in μ_m and J chain mRNA, and the increase in the μ_s/μ_m mRNA ratio only occur in B cells that have virtually completed their differentiation program. B15-TRF, the early acting B cell differentiation factor, although essential for IgM synthesis and secretion, does not, by itself, cause an increase in μ_s or J chain mRNA levels in B cells stimulated with anti-IgM and BSF-p1.

How B15-TRF exerts its action on anti-IgM and BSF-p1–treated B cells is also unresolved. Recently (17), we have shown that molecularly cloned human IL-2, used at high concentration, exerts EL-TRF activity. B cells cultured with anti-

IgM and BSF-p1 display small numbers of IL-2 receptors, as judged by the binding of mAb specific for the T cell IL-2 receptor, and by binding of [^{3}H]IL-2. Treatment of such cells with B15-TRF causes a modest enhancement in receptor number, but whether this change has physiologic significance is not known.

Our results reaffirm the role of IL-2 as a B cell differentiation factor by showing that IL-2, used at 100–1,000 U/ml, causes striking, dose-dependent induction of μ_s and J chain mRNA, a clear increase in the μ_s/μ_m ratio, and the appearance of cells containing cytoplasmic IgM.

In the system we used, 10–30% of the cells cultured with anti-IgM and the three T cell–derived factors possessed cytoplasmic IgM. This suggests that the μ_s/μ_m mRNA ratio in cells actually secreting IgM may be substantially higher than the value of 5.6 obtained in this study. We cannot yet determine the contribution of the cells secreting IgM relative to those not secreting IgM in the μ_s/μ_m ratio, nor can we determine whether the secretory cells express lower μ_m content than the nonsecretory cells. Since 70% or more of the B cells are not synthesizing IgM at a high rate, continued high levels of μ_m RNA in these cells could obscure major changes in the secretory cells.

Similarly, the low levels of μ_s mRNA in B cells treated with anti-IgM and BSF-p1 (or with these stimulants together with B15- or EL-TRF) may reflect either (a) undetectably low levels of cytoplasmic IgM in cells with μ_s, or (b) the presence of very small numbers of IgM-secreting cells that have very high μ_s/μ_m ratios. The change in relative level of μ_s mRNA when all factors are present is of the same order of magnitude as the increase in the percentage of cells with cytoplasmic IgM.

Resting B cells display small amounts of RNA that hybridize with each of the probes used here. Stimulation with anti-IgM and BSF-p1 causes a striking enhancement in μ_m mRNA per cell. However, these changes are much less significant when compared on the basis of constant amounts of RNA, suggesting that the amounts of μ_m mRNA do not increase substantially more than total RNA levels. In support of this are the similar increases in several of the RNA examined, most importantly the mRNA for H-2L^d, a molecule not obviously connected with the B cell activation process.

Finally, our results indicate a very close association between μ_s mRNA and J chain mRNA levels in these cells. This is strikingly illustrated by the time course of amplification of μ_s and J chain mRNA and by their similar dependence on the concentration of IL-2 used as EL-TRF. In addition, the requirement for induction of detectable levels of J chain protein are the same as those required for the appearance of high rate IgM-synthesizing cells, as judged by counting cells with cytoplasmic IgM by immunofluorescent staining. As shown elsewhere, these same requirements hold when the concentration of cytoplasmic IgM is measured by ELISA. Although we have not directly measured IgM secretion, because of the presence of anti-IgM in our cultures, the high levels of μ_s mRNA strongly suggest that a correlation between J chain protein levels and IgM secretion also exists in this system. Thus, this result strongly supports the concept that common regulatory signals are involved in triggering J chain production and IgM secretion in normal B cells (8).

Summary

An increase in mRNA encoding the secretory form of the μ heavy (H) chain (μ_s) and in the ratio of μ_s mRNA to the mRNA encoding the membrane form of the μH chain (μ_m) occurs in normal B cells stimulated with anti-IgM and BSF-p1 together with the two B cell differentiation factors B15-TRF and EL-TRF. Stimulation of cells with anti-IgM and BSF-p1 with either B15- or EL-TRF causes no change in μ_s levels or μ_s/μ_m ratios. The requirements for induction of high rate IgM synthesis in normal B cells stimulated with anti-IgM were precisely the same as those required for elevation of μ_s mRNA levels and for increase in μ_s/μ_m mRNA ratios. A very close correlation also exists between induction of mRNA for J chain and increase in μ_s mRNA levels. Similarly, the increase in J chain protein concentration and percent of cells with cytoplasmic IgM were correlated to each other and to levels of μ_s and J chain mRNA. These results indicate that elevation of μ_s mRNA and μ_s/μ_m mRNA ratios occur in normal B cells only upon commitment to IgM synthesis, and reaffirm the close relation between IgM synthesis and the presence of J chain.

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