DIFFERENTIAL REGULATION OF MEMBRANE AND SECRETORY μ CHAIN SYNTHESIS IN HUMAN B CELL LINES

Regulation of Membrane μ or Secreted μ*

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B cells display distinct patterns of expression of immunoglobulin (Ig) molecules that help to define their specific stage of development. The earliest detectable cell in the B lineage is the pre-B cell. It expresses no surface Ig molecules yet possesses intracytoplasmic μ heavy chains without light chains (sIg^-, $c\mu^+$) (1–4). B lymphocytes are characterized by the production of heavy and light chains resulting in the assembly of monomeric Ig molecules that are detectable on the cell surface (sIg^+) (5). These cells are capable of being triggered by antigen or mitogen to differentiate into plasma cells that secrete large amounts of Ig but express very little surface Ig (2, 6, 7).

It is now evident that, besides phenotypic differences found among B cells at various stages of differentiation, the Ig heavy chain molecule itself changes. Although there is still some controversy (8–10), the membrane-associated heavy chain appears to possess an extra hydrophobic piece at its carboxyterminus. This "tail" is not present on heavy chains secreted by plasma cells, which migrate as slightly smaller molecules on polyacrylamide gels (11–13). The mRNA for these two proteins share the same 5′ sequence. Their differences have been attributed to alternative mechanisms of μ mRNA processing that result in two separate 3′ sequences (14, 15). It is not yet clear whether the heavy chain found in pre-B cells is similar to the membrane μ (μ_m)[1] or secreted μ (μ_s). Results demonstrating secretion by murine fetal liver and human bone marrow pre-B cells (16) suggest that the pre-B heavy chain is more closely related to μ_s Ig heavy chains. However, messenger RNA for both μ_s and μ_m (2.4 kb and 2.7 kb) has been detected in murine fetal liver pre-B cells (4), and both μ_s and μ_m proteins have been isolated from a human pre-B cell line (17).

Recently, tumors and lymphoblastoid cell lines (LBL) with features similar to normal B cells at distinct developmental stages have become available (18). These cells are useful for examining specific B cell characteristics and biosynthetic patterns because large quantities of a relatively pure population of cells can be obtained. While extrapolations to normal cells must be done with great care, the use of LBL and cell

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[1] *Abbreviations used in this paper:* BUdR, 5-bromo-2′-deoxyuridine; ELISA, enzyme-linked immunosorbent assay; LBL, lymphoblastoid cell line; μm, membrane μ; μ_s, secreted μ; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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lines derived from B cell malignancies has permitted the generation of a large amount of data on the biochemical properties of the different species of μ heavy chains (10, 19, 20) and on the DNA sequences encoding the μ chain gene (21–23). However, very little information exists at the molecular level about the mechanisms controlling the synthesis of the various types of μ heavy chain molecules during differentiation.

Previous studies (24, 25) have demonstrated that the thymidine analogue, 5-bromo-2′-deoxyuridine (BUdR), can interfere with the expression of differentiation-specific cell functions without affecting other "housekeeping" functions (24, 25). BUdR is incorporated into the genome in place of thymidine (26) and may interfere with the function of intermediately repetitive DNA sequences (27). We examined the effect of BUdR on Ig expression in several LBL. Our data indicate that BUdR very selectively inhibits the production of μ_m and might allow increased production of μ_s in surface Ig^+ B cell lines; μ chain production by "pre-B" and "plasma" cell lines is unaffected by BUdR incorporation.

Materials and Methods

Cell Lines. The cell lines used in this study possess phenotypes of B cells at various stages of development (Table I). Two lines, Nalm-6 and Daudi, were obtained from Dr. E. W. Ades (Eli Lilly Co., Indianapolis, IN), while others were transformed in this laboratory either spontaneously or with Epstein-Barr virus (LBW 2, 6, 13, 14, 17, and 25) (28, 29). Cell lines were maintained in RPMI 1640 with 10% fetal bovine serum, 2 mM L-glutamine, and 50 μg/ml gentamycin. Cultures were split every 2–4 d, depending on rate of growth.

Incubation with BUdR. 5-bromo-2′-deoxyuridine (Calbiochem-Behring Co., La Jolla, CA) was added to cell cultures at doses ranging from 2.5 to 15 μg/ml for 2–6 d. Initial cell densities ranged from 0.5–1.2 $\times$ 10^6 cells/ml, which usually permitted two cell divisions during the culture period. At the end of the culture period, cells were counted in a Coulter counter, and cell viability was determined by trypan blue dye exclusion. Care was taken to prevent direct exposure of cells to ultraviolet or fluorescent light.

Antibody Reagents and Fluorescent Staining. Goat anti-human μ chain and light chain reagents were affinity purified, as previously described (30). Purity of reagents was determined by (*a*) Ouchterlony gel diffusion, (*b*) immunoelectrophoresis, (*c*) fluorescent staining of specific human myeloma cells, and (*d*) enzyme-linked immunosorbent assays. Conjugation of antibodies to fluorescein isothiocyanate and rhodamine isothiocyanate for surface and cytoplasmic staining were performed as indicated previously (30). Goat anti-human Drw antiserum was a gift from Dr. Marianne Egan (University of Alabama Medical Center, Birmingham, AL).

Radioactive Labeling and Ig Isolation. (*a*) Surface Ig were labeled with ^{125}I by the lactoperoxidase method (31, 32). 10 million cells were labeled, washed extensively with phosphate-buffered saline (PBS), and lysed with PBS containing 0.5% sodium deoxycholate, 0.1% Nonidet P-40, 0.1% NaN_3, 1 mM phenylmethylsulfonylfluoride, 2 mM ϵ-aminocaproic acid, and 1 mg/ml soybean trypsin inhibitor for 30 min at room temperature. Cell lysates were sedimented at 10,000 *g* for 20 min. The 10,000 *g* supernatant was then centrifuged for 60 minutes at 100,000 *g*. This 100,000 *g* supernatant was incubated with either goat anti-human μ, κ, or λ for 90 min at 4°C, then with 50 μl of a 10% suspension of fixed *S. aureus* Cowans I for 30 min at 4°C (33). The precipitate was washed extensively and then treated with sample buffer (20% glycerol, 4% sodium dodecyl sulfate (SDS), 0.12 M Tris-HCl pH 6.8, and 0.005% bromphenol blue) in the presence or absence of 10% 2-mercaptoethanol. The eluted material was separated on 10% SDS-bis-acrylamide gels according to the method of Laemmli (34). (*b*) Cells were biosynthetically labeled by adding 50 μCi of [^{35}S]methionine and cysteine (600 Ci/mmole, Amersham, Arlington Heights, IL) to 1.0–1.5 $\times$ 10^7 control and BUdR-treated cells in 1 ml of RPMI 1640 lacking methionine and cysteine for 6 or 9 h. BUdR-treated cultures were maintained in BUdR during the labeling period. Cells were harvested, culture supernatants were saved, and cells were lysed as above. Ig was isolated from cell lysates and media and labeled material were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as above. Gels were impregnated with

En^{3}Hance (New England Nuclear, Boston, MA), washed, dried, and exposed to Kodak XAR-5 x-ray film (Eastman Kodak Co., Rochester, NY).

Treatment of Cells with Tunicamycin. To distinguish nonglycosylated secretory and membrane μ chains, cultures were treated with 2.5 μg/ml tunicamycin (kindly provided by Dr. R. Hamill, Eli Lilly and Co., Indianapolis, IN) for 90 min before labeling, then continuously during the labeling period. Ig was isolated as above and analyzed after separation on 8–18% SDS-bis-acrylamide gels.

Quantitation of Ig. Ig synthesis was quantitated by either of two methods. Radiolabeled cell lysates, culture supernatants, and surface labeled proteins were cleared and immunoprecipitated as described above and then counted in a Packard 3002 liquid scintillation counter. Nonspecific background radioactivity was subtracted from each sample. Alternatively, cells were analyzed by a quantitative enzyme-linked immunosorbent assay to determine the amount of surface and cytoplasmic μ (35).

Results

Phenotypic Characterization of Cell Lines. Each cell line displayed a phenotype similar to normal cells at specific developmental stages (Table I). Nalm-6 is considered similar to pre-B cells because it produces cytoplasmic μ chains without light chains and lacks surface Ig and Fcγ receptors. The B lymphocyte phenotype is represented by five surface μ^+ cell lines. Some members of this group do not produce light chains, even though they have μ on their surface and express cytoplasmic μ (L. Hendershot and D. Levitt, unpublished results). The plasma cell lines LBW-17 and LBW-25 stain brightly for cytoplasmic μ and light chain but express scant amounts of IgM on their surface. These cells secrete relatively large amounts of pentameric IgM that can be easily detected by both biosynthetic and enzyme-linked immunosorbent assay (ELISA) methods.

Effect of BUdR on Ig Phenotype. When either pre-B or plasma cell lines were incubated with BUdR (0–10 μg/ml) for 3–6 d, no effect on Ig expression could be observed by immunofluorescence (Fig. 1). After 4 d in culture with BUdR, cell lines displaying surface μ exhibited a decrease in the number of surface μ^+ cells in a dose-dependent manner (Fig. 2). This reduction in surface expression of μ was observed in

TABLE I

Characteristics of Lymphoblastoid Cell Lines

Cell Line	Cytoplasmic μ*	Surface μ‡	Light chain*‡	Secretion§	Fcγ‡	Drw‡
NALM-6	+	—	—	—	—	—
LBW-2	++	+	—	—	P	P
LBW-14	++	+	—	—	P	P
DB	++	++	—	—	P	P
LBW-6	++	++	++	—	P	P
LBW-13	++	+	+	—	P	P
DAUDI	+	+++	+++	—	P	P
LBW-17	+++	±	+++	++++	P	P
LBW-25	+++	±	+++	++++	P	P

* Cytoplasmic μ and light chains were detected by fluorescent staining of fixed cytocentrifuge preparations.

‡ Presence of surface antigens was determined by fluorescent staining of intact viable cell suspensions.

§ Secretion of IgM by cell lines was analyzed by quantitative ELISA.

‖ Antigen not detectable (—); dim to very bright (± to ++++); P, antigen present.

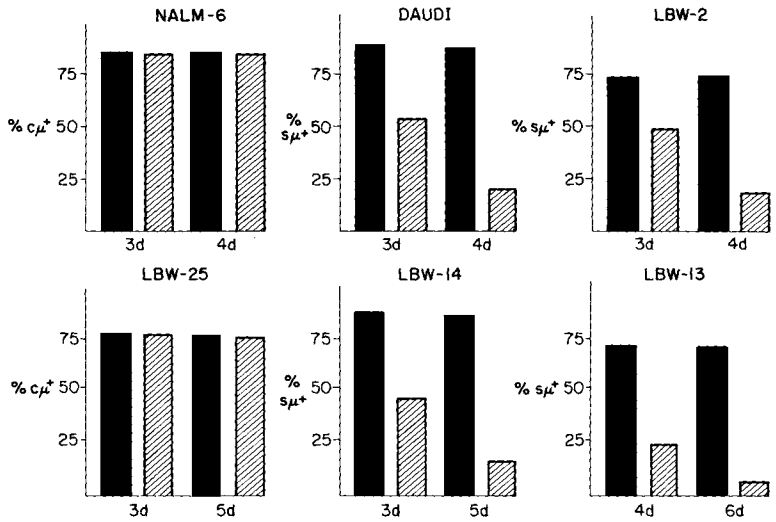

FIG. 1. Effect of BUdR on surface and cytoplasmic expression of μ. Lymphoblastoid cell lines were cultured with 10 μg/ml BUdR for 3–6 d, as noted. Surface μ was detected by fluorescent staining of viable cells, and cytoplasmic μ was observed by fluorescent staining of fixed cytocentrifuge preparations of cultured cells. Both surface and cytoplasmic μ are expressed as the number of positive cells per hundred cells counted. Control (■); BUdR (▨).

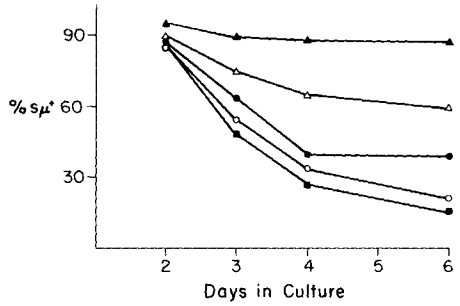

FIG. 2. Dose-response curve for BUdR effect. A surface μ-positive B cell line, LBW-2, was cultured with 0 (▲); 2.5 (△); 5 (●); 7.5 (○); and 10 (■) μg/ml BUdR for 2–6 d. Cells were harvested, and surface μ was detected by fluorescent staining of viable cells with goat anti-human μ.

all surface μ^+ lines regardless of the presence of light chains or cytoplasmic μ (Fig. 1). The loss of surface μ was dependent on cell division and could be blocked by adding equimolar or excess thymidine, but not by adding deoxycytidine (data not shown).

Synthesis of μ in BUdR-treated Cultures. We next examined Ig and total protein synthesis in cells after 4 d of culture in the presence of BUdR. Total protein synthesis (measured by the incorporation of [^{35}S]methionine and cysteine into trichloroacetic acid precipitable counts) was not affected by the incorporation of BUdR into cellular DNA in any of the cell lines examined (Table II). Immunoprecipitation of the labeled cell lysates and supernatants with anti-human μ showed no alteration of Ig synthesis or secretion in pre-B or plasma cell lines after BUdR treatment. When B cell lines were examined, we found that, despite a marked decrease in surface Ig expression, total μ synthesis was not significantly depressed and in some lines actually increased after BUdR incorporation (Table II). These findings were confirmed by quantitating

TABLE II

Quantitation of Ig Synthesis by Radiolabeling and ELISA *

	Radiolabeling cpm $^{35}S/10^6$ cells		ELISA ng $\mu/10^6$ Ig^+ cells		
	Total protein‡	Cell-associated μ§	Total‖	Surface‖	Media‖
Nalm-6					
Control	0.5×10^6	1.7×10^3	98	—	—
BUdR	0.6×10^6	2.1×10^3	95	—	—
LBW-2					
Control	7.8×10^6	6.3×10^4	150	47	10.2
BUdR	8.4×10^6	7.2×10^4	164	20	9.2
Daudi					
Control	6.4×10^6	2.0×10^4	216	120	6.8
BUdR	6.2×10^6	2.8×10^4	196	27	30.2
LBW-17					
Control	3.3×10^6	1.9×10^5	1,360	—	1,720
BUdR	3.4×10^6	2.1×10^5	1,424	—	1,920

* All values represent an average of three experiments.

‡ Total protein synthesis was determined by 10% trichloroacetic acid precipitation of labeled material from cell lysates.

§ Cell-associated IgM production was detected by immunoprecipitation of cell lysates after 9 h. of labeling with [^{35}S]methionine and cysteine.

‖ IgM synthesis was quantitated using the ELISA. Estimation of total cell-associated IgM was performed after lysing cells with detergent buffer. Surface Ig was calculated by an ELISA on intact viable cells. Quantitation of secretion was performed by analyzing day-4 culture supernatants.

total IgM using an ELISA (Table II).

Effect of BUdR on Membrane μ. Daudi cells display easily detectable surface IgM (μ, κ) with little cytoplasmic IgM and appear similar to resting B lymphocytes by immunofluorescent staining. When such cells were treated for 4 d with 10 μg/ml BUdR, the percentage of cells expressing surface IgM decreased, whereas an increase in the number of cells demonstrating cytoplasmic μ by fluorescent staining occurred (Table III). Surface ELISA demonstrated a sixfold decrease in surface IgM but only a slight reduction in total IgM in BUdR-treated cultures (Table II). The loss of ability to synthesize or insert μ_m was most pronounced after stripping surface proteins from control and BUdR-treated Daudi cells with pronase (1.5 mg/ml) (Table III). BUdR-treated cells failed to regenerate surface μ, whereas control cells reexpressed surface IgM within 24 h. The expression of two other surface proteins, Fcγ receptor and Drw antigen, was not reduced qualitatively in BUdR-treated cultures. Unlike surface IgM, these proteins were regenerated after removal by pronase digestion on BUdR-treated cell surfaces (Table III).

Reversibility of BUdR Effects. 85% of Daudi cells failed to exhibit surface IgM after 4 d of culture with BUdR. When these cells were grown for 4 d in the absence of BUdR, the expression of surface μ reached control levels (81%). Thus, it is unlikely that a BUdR-induced mutation is responsible for loss of surface IgM in B lymphocyte cell lines.

SDS-PAGE Analysis of μ Chains Produced by BUdR-treated Cells. Incorporation of BUdR had no effect on the size of μ chain produced by two cell lines, Nalm-6 (pre-B like) and LBW-17 (plasma cell-like), as demonstrated by the isolation and separation of labeled μ chains on SDS-PAGE (Fig. 3). Both cell lines possess cytoplasmic μ chains

TABLE III

*Expression of Surface Markers by Control and BUdR-treated Cells after Pronase Treatment**

	Percent positive cells			
	Surface μ‡	Cytoplasmic μ§	Drw‡	Fcγ receptor‡
Control	90	6	99	99
BUdR	38	12	96	99
20 h after pronase				
Control	86	5	97	<1
BUdR	18	30	96	<1
45 h after pronase				
Control	85	6	96	92
BUdR	10	45	96	95

* Surface markers were stripped by incubating cells in 1.5 mg/ml pronase for 30 min. Cells were washed and allowed to regenerate surface markers. Cells were examined for the expression of surface markers directly after pronase treatment and were found to be negative.

‡ Presence of surface antigens was determined by fluorescent staining of intact viable cell suspensions.

§ Cytoplasmic μ was detected by fluorescent staining of fixed cytocentrifuge preparations.

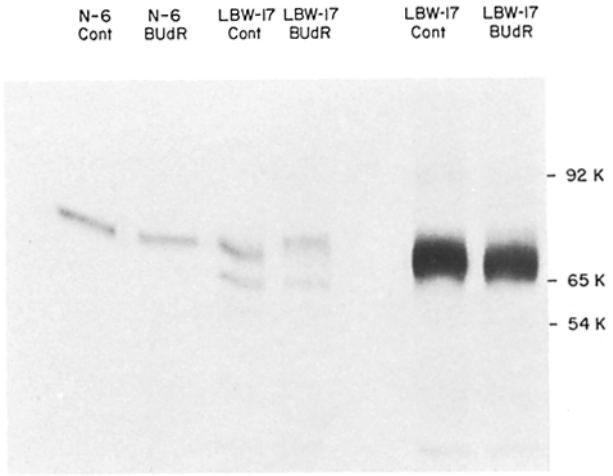

FIG. 3. Ig synthesis by pre-B and plasma cell lines after treatment with BUdR. Nalm-6 and LBW-17 cells were cultured for 4 d with 10 μg/ml BUdR. Control and BUdR-treated cells were then labeled with 50 μCi [^{35}S]methionine and cysteine for 9 h, and IgM was isolated from cell lysates and media. Precipitated material was reduced, alkylated, and separated on 10% SDS-PAGE. Lanes 1–4 are μ chains from lysates; lanes 5–6 are μ chains from media.

that migrate slightly faster than reduced μ chains secreted from LBW-17 or serum IgM standards. Because of the absence of surface μ on either cell line, the secretion of large amounts of pentemeric μ by LBW-17 and an apparent molecular weight of 73

kd for the μ chains isolated from these cells, we are assuming that the cytoplasmic μ chains found in these lines are precursors to μ_s.

IgM produced by BUdR-treated and untreated Daudi cell cultures was next analyzed on polyacrylamide gels. Separation of ^{125}I surface-labeled μ chains from control cells revealed a single strong band with an apparent molecular weight of 78 kd, but only a very faint band could be isolated from the surface of BUdR-treated cells (Fig. 4). After biosynthetic labeling, two sizes of μ chains were precipitated from control cultures (78 kd and 73 kd; Fig. 4, lane 3). The BUdR-treated cultures synthesized significant quantities of only the smaller (73 kd) species of a μ (Fig. 4, lane 4). When control cell lysates were incubated with anti-κ, the 78 kd μ was preferentially precipitated. However, anti-κ precipitation of the BUdR-treated cell lysate clearly precipitated the smaller 73 kd band, demonstrating a shift in light chain association from the 78 kd μ to the 73 kd μ after BUdR treatment (data not shown).

Loss of Higher Molecular Weight μ in BUdR-treated Cultures Is Not Due to Altered Glycosylation. It has recently been demonstrated (11) that the two sizes of μ chain isolated from Daudi cells represent membrane (higher mol wt) and secretory (lower mol wt) μ. Because it is possible that the smaller mol wt μ band synthesized by BUdR-treated cells includes an underglycosylated precursor of μ_m, we analyzed the production of μ chains by control and BUdR-treated Daudi cells in the presence of tunicamycin, an inhibitor of glycosylation (36). Control cultures continued to synthesize two distinct sizes of μ chains, the unglycosylated precursors to μ_m and μ_s (Fig. 5, lane 1). BUdR-treated cells produced only the smaller (pre-secretory) size μ chain (Fig. 5, lane 2). Therefore, the loss of surface μ in B lymphoblastoid cell lines after

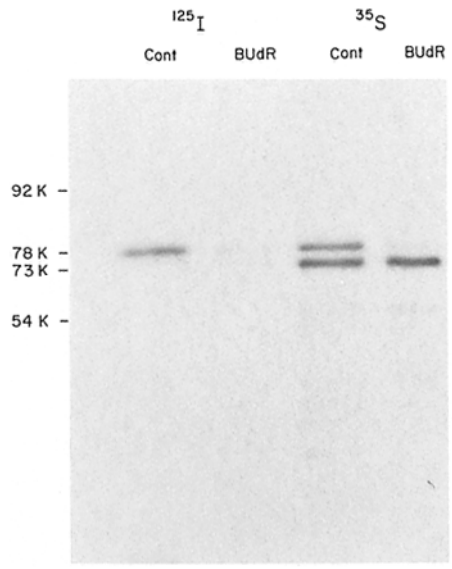

FIG. 4. BUdR blocks μ_m synthesis in B cell lines. 4-d cultures of control and BUdR-treated Daudi cells were either surface labeled with ^{125}I by the lactoperoxidase method or biosynthetically labeled using 50 μCi [^{35}S]methionine and cysteine. Cell lysates were immunoprecipitated with anti-human μ, reduced, alkylated, and separated on 10% SDS-PAGE. μ chains from control (lane 1) and BUdR-treated (lane 2) cells after surface labeling. μ chains from control (lane 3) and BUdR-treated (lane 4) cells after internal labeling.

BUdR treatment is due to the absence of membrane μ production.

Although no IgM could be precipitated from control culture supernatants, small quantities of IgM were isolated from BUdR-treated cells, suggesting that secretion by such cells is possible (data not shown). The μ chain in IgM secreted by BUdR cells co-migrated with the IgM secreted by the LBW-17 plasma cell line.

Discussion

Using the thymidine analogue BUdR (26), we developed a system to evaluate the regulation of μ chain production in human lymphoblastoid cell lines. Incorporation of BUdR into the DNA of pre-B like and plasma cell lines, which probably produce μ_s, failed to inhibit IgM synthesis. However, a clear, reproducible depression of surface μ (μ_m) expression in B lymphoblastoid cell lines was apparent. This blockage occurred even while cytoplasmic μ (μ_s) continued to be expressed in the same B cell line.

Recent information indicates that secretory and membrane μ chains differ in their primary structure at the carboxyterminus (10, 11, 37). The two protein molecules may share a single nuclear transcript that would then be processed differently at the 3′ end (14). It seemed unlikely that incorporation of BUdR into the cellular DNA altered either transcription or translation of the μ chain gene, because both μ_s and μ_m share the same 5′ sequence (14, 38), and only membrane μ production is altered. Instead, it was postulated that either (*a*) BUdR prevented membrane μ proteins from

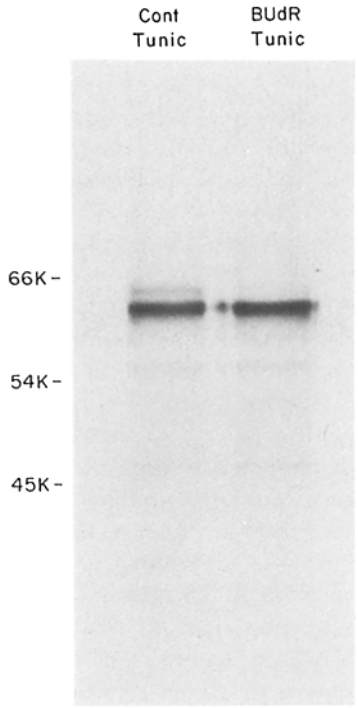

FIG. 5. μ chains produced by control and BUdR-treated B cells in the presence of tunicamycin. 4-d cultures of control and BUdR-treated Daudi cells were preincubated with 2.5 μg/ml of tunicamycin for 90 min, then labeled with [^{35}S]methionine and cysteine for 6 h. Cell lysates were immunoprecipitated with goat anti-human μ, reduced, alkylated, and separated by 8–18% SDS-PAGE.

reaching the surface of B cells, or (*b*) it interfered with the processing of the membrane μ chain message.

Against the former hypothesis (*a*) we found that the expression of other surface proteins, e.g., Fcγ receptors and Drw antigens, was not altered on cells grown in the presence of BUdR. Therefore, the effect of BUdR on μ_m appeared to be relatively selective and not due to a mechanism affecting all membrane proteins.

In support of the latter proposal (*b*) are the biochemical data on μ chain synthesis in control and BUdR-treated cells. Separation of total μ chains from control, surface μ^+ B cell lines revealed two distinct bands; the larger molecule co-migrated with ^{125}I-labeled membrane μ on SDS-PAGE. BUdR-treated cultures, however, produced almost entirely the smaller (73 kd) μ chain. To evaluate whether this smaller μ was a precursor to μ_m and differed simply because of glycosylation (11, 39), we evaluated μ chains produced by control and BUdR-treated cells that were labeled in the presence of tunicamycin (36). Again, two unglycosylated μ chains were discernible in control cultures, and only the smaller μ chain band could be isolated from BUdR-treated cells. Thus, treatment with BUdR inhibited synthesis, and not merely insertion of membrane IgM. Secretory IgM production was unaffected by BUdR.

Because the messenger RNA for μ_s and μ_m are produced by alternative splicing of the same μ gene (15, 38), it is consistent with these data that the effect of BUdR on surface IgM expression is due to interference with μ_m RNA processing but not with μ_s RNA. In fact, processing of μ_s RNA may increase in these lines after μ_m RNA processing is blocked. By two separate methods, we found total IgM production to be unaffected after surface IgM expression was blocked. Although there is an increase in μ_s production after μ_m synthesis is blocked by BUdR, actual secretion of μ chains in these cell lines is not greatly increased. This finding is not surprising because a well-developed microsomal system is necessary for active secretion of virtually any molecule, which BUdR-treated B lymphocyte lines may lack. Further, combination of heavy chains with light chains (40) and J chains (41–43) are also important factors in determining whether an Ig heavy chain is secreted. Of note in this regard is the observation that secretory μ chains can be synthesized by "pre-B cell" hybridomas and cell lines without significant quantities of μ being secreted into the culture medium (44). Therefore, a switch from μ_m processing to μ_s processing would not necessarily result in an increase in IgM secretion. Studies are underway to examine the size and type of μ gene transcript produced in BUdR-treated cells.

The observation that inhibition of μ_m synthesis and expression is reversible in BUdR-treated B lymphoblastoid cells is important. It strongly implies that the mechanisms for regulating the processing of μ chain mRNA can be switched reversibly. The ability to switch mRNA processing from μ_s to μ_m would be necessary if pre-B cells initially synthesize μ_s after V-D-J-C joining (4). Secretory Ig heavy chains might then be synthesized by all cells in the B lineage because they are found even in surface Ig$^+$ B lymphocytes (11, 39). Regulation of mRNA processing during differentiation would primarily involve formation of membrane μ message, either stimulating its appearance during differentiation from a pre-B to B cell or suppressing its production during differentiation from a B cell to plasma cell. Active secretion of Ig molecules would necessitate production of secretory type Ig heavy chains, light chains, possibly J chains, and a well formed endoplasmic reticulum—Golgi complex.

The site of action of BUdR remains unclear. Because it has been previously reported

that low doses of BUdR primarily inhibit "luxury" or differentiation-specific macromolecule expression in many cell types without interfering with normal cellular functions (24, 25), it is unusual that this molecule does not simply halt the production of Ig by cells in the B lineage. Most studies indicate that DNA synthesis (and therefore incorporation of BUdR into the genome) is required for BUdR to exert its effect (26, 45). For membrane μ to be produced, the 5′ end of the membrane exon must be spliced to a 3′ exon of the μ gene that is distinct from the 3′ terminus of μ_s (15). It is possible that BUdR is incorporated into thymidine-rich areas flanking the μ_m exon. Resulting changes in these pyrimidine nucleosides might interfere with the adenylation or splicing of the membrane μ exon. Such alterations could lead to the loss of μ_m RNA production. Alternatively, BUdR may act by changing the production of a splice-adenylation regulator for μ_m mRNA at a site relatively distant from the μ gene (15, 46). By selectively discriminating between the formation of secretory and membrane μ, BUdR will be a useful probe for examining the molecular regulation of these two forms of heavy chains during development, especially when combined with techniques of gene cloning and sequencing.

Summary

Regulation of membrane and secretory μ synthesis was examined in human lymphoblastoid cell lines representing various stages of differentiation. Immunoglobulin phenotype was determined by surface and cytoplasmic staining with fluorochrome-conjugated antibodies and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of anti-μ precipitable cellular products.

The thymidine analogue, 5-bromo-2′-deoxyuridine (BUdR), which inhibits differentiation-specific proteins in a variety of systems, was used to examine regulation of immunoglobulin synthesis. We found that BUdR had a differential effect on membrane (μ_m) and secretory (μ_s) type μ heavy chains. Ig production in pre-B and plasma cell-like lines, which make μ_s, was unaffected by BUdR. However, surface expression of IgM (μ_m) in B cell lines was drastically inhibited at similar doses of BUdR without diminishing total Ig or protein synthesis. Examination of labeled μ chains from control and BUdR-treated B cell lines by SDS-PAGE revealed the production of two sizes of μ (μ_m and μ_s) in control cells and only the smaller size (μ_s) in BUdR-treated cells. This size difference could not be attributed to alterations in glycosylation of the molecules.

These data show that BUdR inhibits the production of membrane μ chains without diminishing secretory μ chain synthesis in the same cell. Our findings suggest that thymidine-rich regions of the genome are involved in the regulation of μ_m vs. μ_s during B cell differentiation.

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