DIFFERENTIAL REGULATION OF MEMBRANE AND SECRETORY \( \mu \) CHAIN SYNTHESIS IN HUMAN B CELL LINES

Regulation of Membrane \( \mu \) or Secreted \( \mu \)*

By LINDA HENDERSHOT AND DANIEL LEVITT

From the Department of Microbiology, University of Alabama in Birmingham, Birmingham, Alabama 35294; and Department of Pediatrics, LaRabida University of Chicago Research Institute Chicago, Illinois 60649

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 \( \mu \) 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 \( \mu \) 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 \( \mu \) (\( \mu_m \))\(^1\) or secreted \( \mu \) (\( \mu_s \)). 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 \( \mu_s \) Ig heavy chains. However, messenger RNA for both \( \mu_s \) and \( \mu_m \) (2.4 kb and 2.7 kb) has been detected in murine fetal liver pre-B cells (4), and both \( \mu_s \) and \( \mu_m \) 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

---

* Supported by grant IN-66T from the American Cancer Society, grants CA 16673 and 5-P01-CA 19266-05 from the National Cancer Institute, and by the Illinois Leukemia Research Foundation.

1 Abbreviations used in this paper: BUdR, 5-bromo-2'-deoxyuridine; ELISA, enzyme-linked immunosorbent assay; LBL, lymphoblastoid cell line; \( \mu_m \), membrane \( \mu \); \( \mu_s \), secreted \( \mu \); PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
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 Χm and might allow increased production of μ 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 × 10⁶ 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 125I 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% NaN₃, 1 mM phenylmethylsulfonylfluoride, 2 mM e-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 [35S]methionine and cysteine (600 Ci/m mole, Amersham, Arlington Heights, IL) to 1.0–1.5 × 10⁷ 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.
En3Hance (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 \( \mu \) chains, cultures were treated with 2.5 \( \mu \)g/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 \( \mu \) (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 \( \mu \) chains without light chains and lacks surface Ig and Fcy receptors. The B lymphocyte phenotype is represented by five surface \( \mu^+ \) cell lines. Some members of this group do not produce light chains, even though they have \( \mu \) on their surface and express cytoplasmic \( \mu \) (L. Hendershot and D. Levitt, unpublished results). The plasma cell lines LBW-17 and LBW-25 stain brightly for cytoplasmic \( \mu \) 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 \( \mu \)g/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 \( \mu \) exhibited a decrease in the number of surface \( \mu^+ \) cells in a dose-dependent manner (Fig. 2). This reduction in surface expression of \( \mu \) was observed in

---

**Table I**

| Cell Line | Cytoplasmic \( \mu^+ \) | Surface \( \mu^+ \) | Light chain*‡ | Secretion§ | Fcγ‡ | Drw‡ |
|-----------|------------------------|------------------|--------------|-----------|------|------|
| NALM-6    | +                      | ~                | ~            | ~         | P    | ~    |
| 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 \( \mu \) 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.
LINDA HENDERSHOT AND DANIEL LEVITT

1625

NALM-6 DAUDI LBW-2

% c~ 50- s/~+% 50 %

st,

50

25- 25 25

3d 4d 5d 4d 3d 4d

LBW-25 LBW-14 LBW-13

% c~ 50-

25-

30-

296x303 Days in Culture

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

90-

% s\( \mu \)* 60

30-

Days in Culture

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

all surface \( \mu^+ \) lines regardless of the presence of light chains or cytoplasmic \( \mu \) (Fig. 1). The loss of surface \( \mu \) 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 \( \mu \) 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 \( \mu \) 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 \( \mu \) 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 | ELISA  |
|---------------|--------|
|               | cpμS/10⁶ cells | ng μ/10⁶ Ig* cells |
|               | Total protein§ | Cell-associated μ§ | Total|| Surface|| Media|| |
| Nalm-6        | 0.5 × 10⁶ | 1.7 × 10⁵ | 98 | — | — | |
| Control       | 0.6 × 10⁶ | 2.1 × 10⁵ | 95 | — | — | |
| BUdR          | 7.8 × 10⁹ | 6.3 × 10⁴ | 130 | 47 | 10.2 | |
| LBW-2         | 8.4 × 10⁹ | 7.2 × 10⁴ | 164 | 20 | 9.2 | |
| Daudi         | 6.4 × 10⁹ | 2.0 × 10⁴ | 216 | 120 | 6.8 | |
| Control       | 6.2 × 10⁹ | 2.8 × 10⁴ | 196 | 27 | 30.2 | |
| BUdR          | 3.3 × 10⁶ | 1.9 × 10⁵ | 1,360 | — | 1,720 | |
| LBW-17        | 3.4 × 10⁹ | 2.1 × 10⁵ | 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 [³⁵S]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 μm 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, Fcy 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‡    | Fcy 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 [35S]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 μE.

IgM produced by BUdR-treated and untreated Daudi cell cultures was next analyzed on polyacrylamide gels. Separation of 125I 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 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 μm, 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 μm and μs (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

![Figure 4](image_url)

**Fig. 4.** BUdR blocks μm synthesis in B cell lines. 4-d cultures of control and BUdR-treated Daudi cells were either surface labeled with 125I by the lactoperoxidase method or biosynthetically labeled using 50 μCi [35S]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 $\mu$ 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 $\mu$ 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 $\mu$ chain production in human lymphoblastoid cell lines. Incorporation of BUdR into the DNA of pre-B like and plasma cell lines, which probably produce $\mu_m$, failed to inhibit IgM synthesis. However, a clear, reproducible depression of surface $\mu$ ($\mu_m$) expression in B lymphoblastoid cell lines was apparent. This blockage occurred even while cytoplasmic $\mu$ ($\mu_c$) continued to be expressed in the same B cell line.

Recent information indicates that secretory and membrane $\mu$ 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 $\mu$ chain gene, because both $\mu_c$ and $\mu_m$ share the same 5' sequence (14, 38), and only membrane $\mu$ production is altered. Instead, it was postulated that either (a) BUdR prevented membrane $\mu$ proteins from

![Figure 5](image-url)
reaching the surface of B cells, or (b) it interfered with the processing of the membrane 
\( \mu \) chain message.

Against the former hypothesis (a) we found that the expression of other surface 
proteins, e.g., Fc\( \gamma \) receptors and Drw antigens, was not altered on cells grown in the 
presence of BUdR. Therefore, the effect of BUdR on \( \mu_m \) 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 \( \mu \) chain synthesis 
in control and BUdR-treated cells. Separation of total \( \mu \) chains from control, surface 
\( \mu^+ \) B cell lines revealed two distinct bands; the larger molecule co-migrated with \( ^{125}\text{I} \)-
labeled membrane \( \mu \) on SDS-PAGE. BUdR-treated cultures, however, produced 
almost entirely the smaller (73 kd) \( \mu \) chain. To evaluate whether this smaller \( \mu \) was a 
precursor to \( \mu_m \) and differed simply because of glycosylation (11, 39), we evaluated \( \mu \) 
chains produced by control and BUdR-treated cells that were labeled in the presence 
of tunicamycin (36). Again, two unglycosylated \( \mu \) chains were discernible in control 
cultures, and only the smaller \( \mu \) 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 \( \mu_m \) and \( \mu_m \) are produced by alternative splicing of 
the same \( \mu \) gene (15, 38), it is consistent with these data that the effect of BUdR on 
surface IgM expression is due to interference with \( \mu_m \) RNA processing but not with 
\( \mu_m \) RNA. In fact, processing of \( \mu_m \) RNA may increase in these lines after \( \mu_m \) 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 
\( \mu_m \) production after \( \mu_m \) synthesis is blocked by BUdR, actual secretion of \( \mu \) 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 mole-
cule, 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 \( \mu \) chains can be synthesized by “pre-B cell” hybridomas 
and cell lines without significant quantities of \( \mu \) being secreted into the culture 
medium (44). Therefore, a switch from \( \mu_m \) processing to \( \mu_m \) processing would not 
necessarily result in an increase in IgM secretion. Studies are underway to examine 
the size and type of \( \mu \) gene transcript produced in BUdR-treated cells.

The observation that inhibition of \( \mu_m \) synthesis and expression is reversible in 
BUdR-treated B lymphoblastoid cells is important. It strongly implies that the 
mechanisms for regulating the processing of \( \mu \) chain mRNA can be switched reversibly. 
The ability to switch mRNA processing from \( \mu_m \) to \( \mu_m \) would be necessary if pre-B 
cells initially synthesize \( \mu_m \) 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 \( \mu \) 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 \( \mu \) to be produced, the 5' end of the membrane exon must be spliced to a 3' exon of the \( \mu \) gene that is distinct from the 3' terminus of \( \mu_c \) (15). It is possible that BUdR is incorporated into thymidine-rich areas flanking the \( \mu_m \) exon. Resulting changes in these pyrimidine nucleosides might interfere with the adenylation or splicing of the membrane \( \mu \) exon. Such alterations could lead to the loss of \( \mu_m \) RNA production. Alternatively, BUdR may act by changing the production of a splice-adenylation regulator for \( \mu_m \) mRNA at a site relatively distant from the \( \mu \) gene (15, 46). By selectively discriminating between the formation of secretory and membrane \( \mu \), 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 \( \mu \) 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-\( \mu \) 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 (\( \mu_m \)) and secretory (\( \mu_s \)) type \( \mu \) heavy chains. Ig production in pre-B and plasma cell-like lines, which make \( \mu_s \), was unaffected by BUdR. However, surface expression of IgM (\( \mu_m \)) in B cell lines was drastically inhibited at similar doses of BUdR without diminishing total Ig or protein synthesis. Examination of labeled \( \mu \) chains from control and BUdR-treated B cell lines by SDS-PAGE revealed the production of two sizes of \( \mu \) (\( \mu_m \) and \( \mu_s \)) in control cells and only the smaller size (\( \mu_s \)) 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 \( \mu \) chains without diminishing secretory \( \mu \) chain synthesis in the same cell. Our findings suggest that thymidine-rich regions of the genome are involved in the regulation of \( \mu_m \) vs. \( \mu_s \) during B cell differentiation.

We thank Dr. M. D. Cooper and Dr. J. F. Kearney for sharing their ideas and helpful criticism of the work, and Dr. W. E. Gathings and P. L. Ho for providing human reagents.

*Received for publication 1 June 1982 and in revised form 18 August 1982.*

**References**

1. Raff, M. C., M. Megson, J. J. T. Owen, and M. D. Cooper. 1976. Early production of intracellular IgM by B lymphocytes precursors in mouse. *Nature (Lond.)* 259:224.
2. Gathings, W. E., A. R. Lawton, and M. D. Cooper. 1977. Immunofluorescent studies of the
development of pre-B cells, B lymphocytes and immunoglobulin isotype diversity in humans. Eur. J. Immunol. 7:804.

3. Burrows, P., M. LeJeune, and J. F. Kearney. 1979. Evidence that murine pre-B cells synthesize μ heavy chains but no light chains. Nature (Lond.). 280:838.

4. Siden, E., F. W. Alt, L. Shirefeld, V. Sato, and D. Baltimore. 1981. Synthesis of immunoglobulin μ-chain gene products precedes synthesis of light chains during B lymphocyte development. Proc. Natl. Acad. Sci. U. S. A. 78:1823.

5. Warner, N. 1974. Membrane immunoglobulins and antigen receptors on B and T lymphocytes. Adv. Immunol. 19:67.

6. Nossal, G. J. V., and B. L. Pike. 1972. Differentiation of B lymphocytes from stem cell precursors. Adv. Exp. Med. Biol. 29:11.

7. Spear, P. G., A.-L. Wang, U. Rutishaiser, and G. M. Edelman. 1973. Characterization of splenic lymphoid cells in fetal and newborn mice. J. Exp. Med. 138:557.

8. Bergman, Y., and J. Haimovich. 1978. B lymphocytes contain three species of μ chains. Eur. J. Immunol. 8:876.

9. Melcher, U., and J. W. Uhr. 1976. Cell surface immunoglobulin. XVI. Polypeptide chain structure of mouse IgM and IgD-like molecules. J. Immunol. 116:409.

10. Williams, P. B., R. T. Kubo, and H. M. Grey. 1978. μ-chains from a nonsecretor B cell line differ from secreted μ-chains at the C-terminal end. J. Immunol. 121:2435.

11. Singer, P. A., H. H. Singer, and A. R. Williamson. 1980. Different species of messenger RNA encode receptor and secretory IgM μ chains differing at their carboxytermini. Nature (Lond.). 285:294.

12. Vassalli, P., J.-C. Jaton, and A. Tartakoff. 1979. Comparison of the biosynthesis and structure of secreted and membrane mouse polyclonal IgM molecules. In B lymphocytes in the Immune Response. M. D. Cooper, D. Mosier, I. Scher, and E. Vitetta, editors. Elsevier/ North-Holland, Amsterdam, Holland. 3.

13. McCune, J. M., V. R. Lingappa, S. M. Fu, G. Blobel, and H. G. Kunkel. 1980. Biosynthesis of membrane-bound and secreted immunoglobulins. I. Two distinct translation products of human μ-chain with identical N-termini and different C-termini. J. Exp. Med. 152:463.

14. Rogers, J., P. Early, C. Carter, K. Calame, M. Bond, L. Hood, and R. Wall. 1980. Two mRNAs with different 3′ ends encode membrane-bound and secreted forms of immunoglobulin μ chains. Cell. 20:303.

15. Early, P., J. Rogers, M. Davis, K. Calame, M. Bond, R. Wall, and L. Hood. 1980. Two mRNAs can be produced from a single immunoglobulin μ gene by alternative RNA processing pathways. Cell. 20:313.

16. Levitt, D., and M. D. Cooper. 1980. Mouse pre-B cells synthesize and secrete μ heavy chains but not light chains. Cell. 19:617.

17. McCune, J. M., and S. M. Fu. 1981. Ig biosynthesis in a human pre-B cell line. J. Immunol. 127:2609.

18. Cantor, H., and E. A. Boyse. 1977. Lymphocytes as models for the study of mammalian cellular differentiation. Immunol. Rev. 33:105.

19. Singer, P. A., and A. R. Williamson. 1980. Cell surface immunoglobulin μ and γ chains of human lymphoid cells are of higher apparent molecular weight than their secreted counterparts. Eur. J. Immunol. 10:180.

20. Vassalli, P., R. Tedghi, B. Lisowska-Bernstein, A. Tartakoff, and J. Jaton. 1979. Evidence for hydrophobic region within heavy chains of mouse B lymphocyte membrane-bound IgM. Proc. Natl. Acad. Sci. U. S. A. 76:5525.

21. Brack, C., M. Hirama, R. Lenhard-Schuller, and S. Tonegawa. 1978. A complete immunoglobulin gene is created by somatic recombination. Cell. 13:1.

22. Davis, M., K. Calame, P. W. Early, D. L. Livant, R. Joho, I. L. Weissman, and L. Hood. 1980. An immunoglobulin heavy chain gene is formed by at least two recombinational
events. *Nature (Lond.)*. 283:733.

23. Liu, C. P., P. W. Tucker, J. F. Mushinski, and F. Blattner. 1981. Mapping of heavy chain genes for mouse immunoglobulins M and D. *Science (Wash. D. C.)*. 209:1348.

24. Holtzer, H., and J. Abbott. 1968. Oscillation of the chondriogenetic phenotype in vitro. In *The Stability of the Differentiated State*. H. Ursprung, editor. Springer-Verlag, New York.

25. Preisler, H. D., D. Housman, W. Scher, and C. Friend. 1973. Effects of 5-bromo-2'-deoxyuridine on production of globin mRNA in dimethylsulfoxide-stimulated Friend leukemia cells. *Proc. Natl. Acad. Sci. U. S. A.* 70:2956.

26. Rutter, W. J., R. L. Pictet, and P. W. Morris. 1973. Toward molecular mechanisms of developmental processes. *Annual Rev. Biochem.* 42:501.

27. Strom, C., and A. Dorfman. 1976. Distribution of 5-bromodeoxyuridine and thymidine in the DNA of developing chick cartilage. *Proc. Natl. Acad. Sci. U. S. A.* 73:1019.

28. Henle, W., V. Diehl, G. John, H. zur Hausen, and G. Henle. 1967. Herpes-type virus and chromosome markers in normal leukocytes after growth with irradiated Burkitt cells. *Science (Wash. D. C.)*. 157:1064.

29. Epstein, M. A., and B. G. Achong, editors. 1979. *In The Epstein-Barr Virus*, Springer-Verlag, New York. 205.

30. Kearney, J. F., and A. R. Lawton. 1975. B lymphocyte differentiation induced by lipopolysaccharide. I. Generation of cells synthesizing four major immunoglobulin classes. *J. Immunol.* 115:671.

31. Marchalonis, J. J. 1969. An enzymic method for the trace iodination of immunoglobulins and other proteins. *Biochem. J.* 113:299.

32. Phillips, D. R., and M. Morrison. 1970. The arrangement of proteins in the human erythrocyte membrane. *Biochem. Biophys. Res. Commun.* 40:284.

33. Kessler, S. W. 1975. Rapid isolation of antigens from cells with a staphylococcal protein A antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. *J. Immunol.* 115:1617.

34. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*. 227:680.

35. Ho, P.-L., and D. Levitt. 1982. A rapid method for quantitation of cell surface IgM by an enzyme-linked immunosorbent assay. *J. Immunol. Methods*. In press.

36. Lehle, L., and W. Tanner. 1976. The specific site of tunicamycin inhibition in the formation of dolichol-bound N-acetylglucosamine derivatives. *FEBS Lett.* 71:167.

37. Kehry, M., S. Ewald, R. Douglas, C. Sibly, W. Raschke, D. Fambrough, and L. Hood. 1980. Membrane-bound and secreted forms of immunoglobulin /x chain differ at their carboxytermini. *Cell.* 21:393.

38. Alt, F. W., A. L. M. Bothwell, M. Knapp, E. Siden, E. Mather, M. Koshland, and D. Baltimore. 1980. Synthesis of secreted and membrane-bound immunoglobulin mu heavy chains is directed by mRNAs that differ at their 3' ends. *Cell.* 20:293.

39. Sidman, C. 1981. B lymphocyte differentiation and the control of IgM /x chain expression. *Cell.* 23:379.

40. Weitzman, S., D. Marguilis, and M. Scharff. 1976. Mutations in mouse myeloma cells: implications for human multiple myeloma and the production of immunoglobulins. *Ann. It. Med.* 85:110.

41. Koshland, M. E. 1975. Structure and function of the J chain. *Adv. Immunol.* 20:41.

42. Roth, R. A., E. L. Mather, and M. E. Koshland. 1979. Intracellular events in the differentiation of B lymphocytes to pentamer IgM synthesis. In *Cells of Immunoglobulin Synthesis*. H. Vogen and B. Pernis, editors. Academic Press, Inc., New York. 141.

43. Mather, E. L., F. W. Alt, A. L. M. Bothwell, D. Baltimore, and M. E. Koshland. 1981. Expression of J chain RNA in cell lines representing different stages of B lymphocyte
differentiation. Cell. 23:369.

44. Perry, R. P., D. E. Kelley, C. Coleclough, and J. F. Kearney. 1981. Organization and expression of immunoglobulin genes in fetal liver hybridomas. Proc. Natl. Acad. Sci. U. S. A. 78:247.

45. Levitt, D., and A. Dorfman. 1974. Concepts and mechanisms of cartilage differentiation. In Current Topics in Developmental Biology. A. Moscona and A. Monroy, editors. Academic Press, Inc., 8A:103.

46. Rogers, J., and R. Wall. 1980. A mechanism for RNA splicing. Proc. Natl. Acad. Sci. U. S. A. 77:1877.