Differentiation of Chronic Lymphocytic Leukemia Cells: Correlation Between the Synthesis and Secretion of Immunoglobulins and the Ultrastructure of the Malignant Cells

By A. Rubartelli, R. Sitia, A. Zicca, C. E. Grossi, and M. Ferrarini

The capacity of synthesizing and secreting Ig molecules was studied in 11 patients with B-cell chronic lymphocytic leukemia (B-CLL) whose cells expressed surface IgM, in 3 patients with surface IgG-bearing cells, and in 2 IgM prolymphocytic leukemias (IgM-PLL). Three types of μ chains were detected by SDS-polyacrylamide gel electrophoresis analysis of the endogenously labeled molecules isolated by specific immunoprecipitation. Two of them were isolated from the cell lysates and were identified as the membrane μ chain and the precursor of the secreted molecules, respectively. The latter also possibly contained precursors of the membrane molecules. The third type of molecule was detected only in the culture medium and was identified as secretory μ chain. Not all of the malignant clones possessed the three types of μ chains. Only ⅓ of the IgM-bearing malignant cell clones were capable of secretion, whereas the remaining synthesized the secretory μ chains but degraded them intracellularly. Two types of molecules (membrane and secreted) were found in the IgG-bearing CLL cells from three patients. In all of them, secretion was detected. Ultrastructural analysis demonstrated that cells from the secreting clones had the features of more mature lymphocytes than the cells from nonsecreting clones. These features were represented by a developed Golgi apparatus, various types of vesicles (smooth and coated), and strands of the rough endoplasmic reticulum. A certain heterogeneity of the degree of maturation of the cells was observed within these clones. The data are consistent with the hypothesis that CLL clones are heterogeneous and can be distinguished through the different degrees of maturation of their cell components.

LYMPHOPROLIFERATIVE disorders are due to the clonal expansion of lymphoid cells. Traditionally, the malignant cells of these disorders have been considered as “frozen” at a given maturational stage, with the remarkable exception of chronic lymphocytic leukemia (CLL) with serum monoclonal components and Waldenström's macroglobulinemia, where a process of maturation induces the transformation of some lymphocytes of the malignant clone into secreting plasma cells. Recent immunochemical, cytochemical, and ultrastructural studies have provided evidence favoring the hypothesis that a process of maturation, although limited, may occur in other lymphoproliferative disorders. The process of B-lymphocyte differentiation toward plasma cells requires that the cells secrete Ig. Therefore, secretion of Ig may be taken as a marker for B-cell maturation. When studies with such a marker are carried out, biochemical techniques that analyze the Ig produced seem particularly suitable, since membrane and secreted Ig can be differentiated by the variations in their size and in the primary structure of their heavy chain. The C-terminus of the membrane heavy chain is hydrophobic in order to fulfill its role of anchoring the immunoglobulin to the membrane, whereas the heavy chain of the secreted molecule has a shorter, hydrophilic C-terminus peptide.

In this study of a number of patients with B-cell lymphoproliferative disorders, the malignant cells that expressed membrane IgM or IgG (as detected by immunofluorescence) have been analyzed for their capacity to synthesize and secrete Ig molecules. In most cases, Ig secretion was observed, whereas in a minority of patients, precursors of secreted Ig were degraded intracellularly. Ultrastructural analyses of the leukemic cells revealed differences among the various clones and within the cell components of each single clone. Generally, the capacity to secrete Ig was related to the presence within the clone of a number of cells with ultrastructural characteristics of maturation, such as an extended Golgi apparatus, strands of the rough endoplasmic reticulum (RER) and several types of smooth and coated vesicles. These data support the view that a process of intraclonal maturation occurs in a number of B-cell malignancies and provide a new insight into the complex biochemical steps involved in the process of B-cell differentiation.

MATERIALS AND METHODS

Patients

Fourteen patients with B-CLL and two with prolymphocytic leukemia (PLL) were examined. The diagnosis was confirmed by clinical and histologic criteria. All of the patients had a white blood cell count ranging from 20 to 100 x 10³/cu mm (80%-95%
lymphocytes). Some of the features of the patients, together with the surface markers of the malignant cells, are summarized in Table 1.

**Antisera**

Goat anti-human Ig (polyvalent) and anti-γ, μ, δ, and α antisera were prepared, and the F(ab)₂ fragments conjugated with fluorescein isothiocyanate (FITC) (BBL, Cockeysville, MD), as previously described.

Monoclonal antibodies to human μ chains were a kind gift of Dr. A. Bargellesi (Department of Biochemistry, University of Genoa). Monoclonal antibodies to κ and λ (BAM03 and BAM04) were purchased from Seward Laboratory (Bbraco, Milano, Italy).

An antiidiotype antiserum against the IgGk M-component of one patient was raised in a rabbit by repeated injections of the monoclonal protein, purified from the serum by DEAE chromatography, in complete Freund’s adjuvant. The antiserum was absorbed extensively with human erythrocytes and normal human serum, cord serum, and purified IgM or IgG myeloma proteins, all made insoluble with Sepharose 4B. The specificity of the antiserum for the monoclonal protein was demonstrated by: (1) the lack of reactivity with monoclonal IgG, IgM, and IgA or normal human serum in double immunodiffusion; (2) the failure to stain IgM- or IgG-bearing cells from CLL patients and from normal human tonsil; and (3) the failure to precipitate immunoglobulin from other radiolabeled CLL cells as well as from normal B lymphocytes.

**Isolation of Peripheral Blood Mononuclear Cells and Marker Analysis**

Mononuclear cells were isolated by Ficoll-Hypaque gradients from the heparinized blood of the patients. Cells forming rosettes with neuraminidase-treated sheep red cells (E refractile) were measured as previously described. Surface immunoglobulin (slg) and intracytoplasmic immunoglobulin (clg) were detected by direct immunofluorescence with anti-class or anti-type-specific reagents.

**Endogenous Labeling**

Cells were washed 3 times in methionine or leucine-free RPMI 1640 medium, resuspended at 10 x 10⁶/ml in 1 ml of the same medium supplemented with 5% fetal calf serum (FCS) that was previously dialyzed against PBS, 200 U/ml penicillin, 200 μg/ml streptomycin, 2 mM glutamine, 1 mM sodium pyruvate, and 5 x 10⁻⁵ M 2-mercaptoethanol (2ME). ¹²⁵I-Leucine (specific activity 350 mCi/mmol) and ¹³⁵S-methionine (specific activity >800 Ci/mmol) were purchased from New England Nuclear (Firenze, Italy) and added at a concentration of 20–50 μCi/ml. Cells were labeled for 12 hr in 5% CO₂ at 37°C. In the pulse-chase experiments, 35 x 10⁶ cells were first incubated for 1 hr at 37°C in methionine-free RPMI 1640 supplemented with 5% dialyzed FCS and subsequently incubated for 15 min in 0.5 ml of the same medium containing 400 μCi ³⁵S-methionine. Labeled cells were chased at the concentration of 7 x 10⁶ cells/ml in RPMI 1640 containing 10% FCS for 1, 3, 6, or 20 hr. A quantity of 7 x 10⁶ labeled cells was not chased and was used as the time 0 sample.

At the end of the incubation period, the cells were centrifuged, washed 3 times in cold PBS, and lysed in PBS containing 0.5% Nonidet P40. Protein-bound radioactivity present both in the cell lysates or in the culture supernatants was measured by trichloroacetic acid precipitation.

**Membrane Radiiodination**

Membrane radioiodination was carried out according to a modification of the technique of Marchalonis et al. Briefly, 20 x 10⁶ viable cells were washed 4 times in PBS and resuspended in 50 μl of PBS; subsequently 100 μl of 1 mg/ml solution of lactoperoxidase (Sigma, St. Louis, MO), and 0.5 mCi of carrier-free ¹²⁵I (Sorin, Vercelli, Italy) were added. Fifty microliters of 0.03% H₂O₂ was added 3 times at 3-min intervals. Labeled cells were washed 3 times in cold PBS and lysed with Nonidet P40 as above.

**Immunoprecipitation**

Samples, radiolabeled as described above, were first incubated with normal rabbit serum coupled to Sepharose 4B (Pharmacia, Uppsala, Sweden) in order to remove any protein that could bind nonspecifically to the immunoadsorbent. The samples were then incubated with 50 μl of Sepharose-bound specific antisera or Sepharose-bound tireoglobulin at 4°C, on rotation for 2 hr.

**Table 1. Principal Immunologic Features of the Patients**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Surface Ig*</th>
<th>Cytoplasmic Ig†</th>
<th>Serum M-Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL1</td>
<td>IgM</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A</td>
<td>IgM</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>IgM</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C</td>
<td>IgM, IgM</td>
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<td>D</td>
<td>IgM, IgM</td>
<td>—</td>
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<tr>
<td>E</td>
<td>IgM, IgD</td>
<td>—</td>
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<tr>
<td>F</td>
<td>IgM, IgD</td>
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<td>G</td>
<td>IgM, IgM</td>
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<td>H</td>
<td>IgM, IgD</td>
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<tr>
<td>I</td>
<td>IgM</td>
<td>2% IgM</td>
<td>IgM</td>
</tr>
<tr>
<td>L</td>
<td>IgM, IgG</td>
<td>2% IgM, 0.5% IgG</td>
<td>IgM, IgG</td>
</tr>
<tr>
<td>M</td>
<td>IgM</td>
<td>3% IgM, 1% IgG</td>
<td>IgM, IgG</td>
</tr>
<tr>
<td>N</td>
<td>IgG</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>O</td>
<td>IgG</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P</td>
<td>IgG</td>
<td>3% IgG</td>
<td>IgG</td>
</tr>
<tr>
<td>PLL</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Q</td>
<td>IgM</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>R</td>
<td>IgM</td>
<td>—</td>
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</tr>
</tbody>
</table>

*As determined by immunofluorescence on viable cell suspensions. In each case, >80% of the cells stained with the specific reagents, with the exception of case L. In this patient, 10% of the cells stained for γ and 80% for μ.
†As determined by immunofluorescent staining of cytocentrifuge preparations after fixation.
‡Type II, mixed, essential cryoglobulinemia. ¹⁶, ¹⁷
Alternatively, samples were immunoprecipitated with 5 μg of a specific antibody (or of a control reagent) followed by 50 μl of protein A coupled to Sepharose (PAS). The anti-IgG activity of the labeled molecules from the cryoglobulinemia patient cells was studied by incubating cell lysates and supernatants with Sepharose-bound human IgG. Immunoprecipitates were washed 3 times with PBS containing 0.5% Nonidet P40, 10 mM EDTA, 1 mM phenyl methyl sulfonyl fluoride, 10 mM iodoacetamide, and 1 M NaCl, once with 20 mM Tris-HCl, pH 6.8, and subsequently eluted by boiling in Laemmli stacking buffer containing 2% SDS and 5% 2ME for 2 min.

**Sodium Dodecyl Sulfate (SDS) Polyacrylamide Gel Electrophoresis**

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was performed by a modification of the method of Laemmli, as previously described. Gels were dried and autoradiographed on 3M XD films (Ferrania 3M, Genova, Italy), with or without intensifying screen. Fluorography was used to amplify 14C- and 35S-labeled gels. 14C marker proteins, including phosphorylase B (92.5 kd), bovine serum albumin (69 kd), and carbonic anhydrase (30 kd), were obtained from NEN (Firenze, Italy).

**Inhibition of Glycosylation**

The antibiotic tunicamycin (Sigma, St. Louis, MO) was used to inhibit N-linked glycosylation of asparagine residues. Cells were incubated with 5 μg/ml of tunicamycin for 1 hr before the addition of labeled amino acids. Previous studies with radiolabeled sugars demonstrated that more than 95% Ig glycosylation was inhibited by tunicamycin at a concentration greater than 1 μg/ml.

**Electron Microscopy**

Cell suspensions were fixed with 1.25% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.6, at room temperature for 30 min. Following extensive washing in cacodylate, cells were postfixed in 1% osmium tetroxide in the same buffer at room temperature for 30 min, dehydrated with ethanol, and embedded in Agar 100 (Agar Aids, Stansted, Essex, England) resin. Thin sections were stained with uranyl acetate and lead citrate and examined under a Siemens Elmiskop 101 transmission electron microscope.

**RESULTS**

**Endogenous Labeling of Ig Produced by Malignant B Cells**

Cell lysates and culture fluids of endogenously labeled CLL cells were immunoprecipitated with anti-μ and anti-γ antisera or with tiroglobulin, and the immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis under reducing conditions (Fig. 1 and Table 1).

Cell lysates of IgM-bearing CLL contained two anti-μ precipitable bands, displaying an apparent molecular weight of 83 ± 4 and 79 ± 4 kd, respectively. The variations in the apparent molecular weight were not only attributable to the technique employed but also to minor differences consistently observed among the single CLL cases. The faster moving μ chain was present in all of the cases, whereas the other was evident in 7, but barely visible or nondetectable in the remaining cases. A μ chain of an apparent molecular weight of 81 ± 4 was detected in the culture fluids of 6 of the cases. These included all of the patients with a serum IgM monoclonal component. Two μ chain bands were detected in the cell lysates of the two PLL studied. In one case, the apparent molecular weight of the chains was of 80 and 76 kd, respectively, whereas the values found in the other case were 82 and 78 kd. The culture supernatants of the latter case also displayed an 81-kd μ chain, which was not seen in the other patient. No bands were detected in the SDS-polyacrylamide gel electrophoresis in the control (ti-

![Fig. 1](https://www.bloodjournal.org/bloodjournal/2017/11/15/fig1.png)

Fig. 1. Radioautography of a 10% SDS-polyacrylamide gel electrophoresis run under reducing conditions. The different biosynthetic patterns of 3 cases of IgM-CLL, 2 of IgM-PLL, and 1 of IgG-CLL are shown (left to right). Anti-μ and anti-γ immunoprecipitates were obtained from cell lysates (cl) or tissue culture supernatants (s) of cells endogenously labeled for 12 hr in the presence of 50 μ Ci 35S-methionine. The different gels were aligned according to the molecular weight markers. Cl and s from the same patient cells were always electrophoresed in the same gel.)
shown). Two γ chains of an apparent molecular weight of 63 and 55 kd could be isolated from the cell lysates of the three IgG-bearing CLL. A single γ chain displaying the same apparent molecular weight as the faster moving intracytoplasmic γ chain was detected in the culture supernatants. Again, no bands were observed in the SDS-polyacrylamide gel electrophoresis of the control or of the anti-μ immunoprecipitates (not shown).

In the two cases displaying two distinct serum M-components, IgMx and IgGx, both μ and γ chains were isolated, either from cell lysates or supernatants. Table 2 summarizes all of the findings of the endogenous labeling experiments in the different cases. That the labeled molecules were produced by the malignant cells and not by contaminating normal B cells was demonstrated as follows:

(1) Labeled samples were divided into two tubes and precipitated with anti-κ or anti-λ antisera. As expected from the results of immunofluorescence, an amount of radioactivity significantly above the control values was consistently precipitated by one or the other of the two reagents. Likewise, heavy and light chain bands were detected by SDS-polyacrylamide gel electrophoresis in one of the two anti-light chain immunoprecipitates only.

(2) An antiidiotype antiserum was raised against the serum monoclonal IgG of one CLL patient (case P in Table 1). This reagent precipitated the same amount of radioactivity from both the radiolabeled cell lysates and culture supernatants as the anti-γ antisera. Following precipitation with the antiidiotype antiserum, no additional radioactivity was precipitated with the anti-γ reagent. Furthermore, SDS-polyacrylamide gel electrophoresis analysis of the antiidiotype immunoprecipitates demonstrated that the antiserum reacted with IgG in both of the molecular forms described above.

(3) The cells from patient I (see Table 1), who had mixed essential (type II) cryoglobulinemia, were endogenously labeled in vitro. The cell lysates and culture supernatants were reacted with Sepharose-bound human IgG. SDS-polyacrylamide gel electrophoresis analysis of the immunoprecipitates showed a single μ chain (apparent molecular weight of 80 kd) in the cell lysates and another μ chain (apparent molecular weight of 82 kd) in the culture supernatants. The same SDS-polyacrylamide gel electrophoresis pattern was observed when both cell lysates and culture supernatants were precipitated with anti-μ antisera. By contrast, following the reaction with insolubilized IgG, no additional molecules were precipitated by the anti-μ reagent, indicating that all of the molecules produced by the malignant clone had anti-IgG activity. No precipitate was obtained with insolubilized IgG from the lysates or the culture supernatants from five other cases (data not shown).

Radioiodination of Membrane Ig

IgM-bearing cells from patients H and R (Table 1) and IgG-bearing cells from patient O were membrane radioiodinated and the labeled Ig immunoprecipitated and analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 2). The precipitates showed only one form of

![Fig. 2. Radioautography of a 10% SDS-polyacrylamide gel electrophoresis run under reducing conditions. Anti-μ and anti-γ immunoprecipitates from 125I radioiodinated (lanes 1 and 3) and endogenously labeled (lanes 2 and 4) cells from one IgM-CLL and one IgG-CLL, respectively.](image-url)
either \( \mu \) or \( \gamma \) chain displaying the same apparent molecular weight as the slow migrating \( \mu \) and \( \gamma \) chains detected in the cell lysates of endogenously labeled cells. This finding shows that the slow migrating heavy chains represented the membrane form of IgM (\( \mu_m \)) or IgG (\( \gamma_m \)). The failure to detect \( \mu \) molecules by the endogenous labeling techniques in some CLL cases (Table 2) is consistent with the finding of the faint staining of the cells by immunofluorescence.

Identification of the Nonglycosylated Precursor of the Heavy Chains

The faster moving heavy chain band detected in the above endogenous labeling experiments could represent the precursor of the membrane, of the secreted heavy chain, or of both. In order to investigate this problem and to clarify whether or not the malignant cells were capable of synthesizing more than one polypeptide backbone of the heavy chain, cells from cases F (IgM+ CLL) and R (IgM+ PLL) were incubated in the presence tunicamycin, a drug that inhibits N-linked glycosylation.\(^{23,24}\)

As shown in Fig. 3, two intracytoplasmic bands were detected. One slow moving band of apparent molecular weight 66 kd was detected in the cell lysates only, whereas both the cell lysates and culture supernatants contained a faster moving chain of an apparent molecular weight of 64 kd. These data indicate that two peptide \( \mu \) chains were synthesized, the heavier of which probably represented the \( \mu_m \) precursor and the lighter the precursor of the secreted \( \mu \) chain (\( \mu_i \)).

Pulse-Chase Experiments

A possible precursor–product relationship among the faster moving intracytoplasmic \( \mu \) chain (\( \mu_c \)), the \( \mu \), and the \( \mu_m \) molecules was investigated by pulse-chase experiments. IgM-bearing cells from two CLL patients (cases B and G) and one PLL patient (case R) were pulsed with \( ^{35} \)S-methionine for 15 min and then chased in cold medium. The SDS-polyacrylamide gel electrophoresis of the \( \mu \) chains in the lysates and supernatants at different times are shown in Fig. 4.

Three different patterns were observed: in one case (patient B) that had \( \mu_m \) only, a single \( \mu_c \) band was heavily labeled immediately after the pulse and its intensity decreased during the chase period. No \( \mu \) chains were detected in the culture fluids. In another
case (patient G) that had $\mu_c$ and $\mu_n$, the same pattern was observed with the exception that a $\mu$ molecule appeared in the culture supernatants after the first hour of chase and its intensity increased thereafter. In the third case (patient R) that had $\mu_c$, $\mu_n$, and $\mu_s$, a $\mu_c$ band only was detected after the pulse period, whereas a $\mu_s$ and a $\mu_n$ band appeared in the supernatants and in the lysates, respectively, after 1 hr of chase. The $\mu_c$ band progressively became more evident, whereas the intensity of the $\mu_n$ band decreased. By contrast, no particular changes were seen in the intensity of the $\mu_s$ band. This is probably due to the different turnover rates of the two molecules.

The above data show that the $\mu_c$ chain was the first molecule to be synthesized. In some instances, this molecule was degraded intracellularly, whereas in others it was processed progressively to be secreted in the form of $\mu_n$ chain. In the absence of any other detectable intracytoplasmic $\mu$ chain, it is conceivable that the $\mu_c$ band also comprised the precursor molecule of $\mu_n$.

Similar results were obtained with the IgG-bearing cells from the three CLL cases studied (patients N, O, P), where secretion was always observed. Conversion of $\gamma_c$ to $\gamma_s$ was observed (Fig. 5). The membrane $\gamma$ chain band was detected immediately after the pulse period and began to decrease in its intensity after 3 hr of chase.

**Ultrastructural Features of the Malignant Cells**

Previously, it was reported that three major cell types could be classified as early (type 1), intermediate (type 2), or late (type 3) stages of B-cell differentiation.

Type 1 cells were characterized by the absence of cytoplasmic membrane systems, the only organelles present being scarce mitochondria, centrioles, and monoribosomes within a relatively scanty cytoplasm (Fig. 6A).

Type 2 cells had an extended cytoplasm with prominent smooth membrane systems. The smooth membranes were assembled to form an extended Golgi apparatus and a variety of smooth vesicles. The Golgi apparatus was located in the proximity of the nucleus and consisted of several stacks of usually empty smooth cisternae (Fig. 6B). At least three types of vesicles could be identified, i.e., coated vesicles, cup-shaped vesicles, and matrix-containing vesicles. The coated vesicles were near the trans aspect of the Golgi apparatus and had a thick wall with a fuzzy coat on their outer side (Fig. 7A). The cup-shaped vesicles were similar to those of other cells, including lymphocytes, were located near to the Golgi zone, and appeared void of any content. They had a variable size and were bounded by a membrane that was thin and sometimes discontinuous at the concave side and thick at the convex side (Fig. 7B). The matrix-containing vesicles (Fig. 7C) had a round shape, a variable size, and contained a flocculent material of low electron opacity. Type 2 cells had very little or no RER. When present, this consisted of short isolated strands.

Type 3 cells (Fig. 6C) were characterized by the presence of long strands of RER that appeared to be either isolated or arranged into parallel arrays. These cells also possessed an extended Golgi apparatus and
Fig. 6. Ultrastructural features of representative cells from patients with predominant type 1 (A), type 2 (B), and type 3 (C) cells. Note the progressive development of the Golgi apparatus and of the rough endoplasmic reticulum. (D) The ultrastructure of a cell from patient Q with prolymphocytic leukemia. (A–D. x 5,000.)

numerous vesicles, particularly of the coated and of the matrix-containing type.

Patients with type 1 cells usually had >90% of this type of lymphocyte, the remaining cells belonging mostly to type 2. Patients with predominant cells of the other types displayed a higher degree of morphological heterogeneity, so it was sometimes difficult to classify a number of cases as type 2 or type 3 (see Table 2). In agreement with the immunofluorescence data, the cases with serum M-components had occasional cells with the fine structure of mature plasma cells.

As shown in Fig. 6D, the cells from the two PLL patients had the typical features of prolymphocytes, with irregularly shaped nuclei containing large nucleoli. Cells from patient R had an extended cytoplasm with a large Golgi apparatus, numerous strands of RER, and a variety of vesicles. These features were less evident in cells from patient P.

DISCUSSION

Cells from 10 out of 14 CLL patients and one out of two PLL patients secreted Ig molecules in vitro. These Ig were not shed from the cell membrane, since identical molecules were also consistently found in the
supernatants of the cells from patients with serum M-components and circulating plasma cells. More important, these molecules had an apparent molecular weight different from that of the membrane Ig. In agreement with previous data,\(^9,15\) such differences in the apparent molecular weight could be in part related to a different heavy chain polypeptide structure, as shown by tunicamycin studies.\(^23,24\) The results of the pulse-chase experiments also support the view that CLL cells were capable of secreting Ig molecules.

The ultrastructural data were largely in agreement with the biochemical studies in that there was a correlation between the secretory capacity of the cells and their degree of maturation determined by morphological criteria. Cells from the 11 CLL patients examined by EM were classified as predominant type 1 in 4 cases. A lack of Ig secretion was observed in 3/4 of these cases. The single exception found in this group of patients can be explained by the presence within the malignant clone of a minority of mature (type 2 or 3) B cells or, alternatively, by the possibility that, under special circumstances, small resting B lymphocytes possess a secretory capacity.\(^31\) Cells from the remaining 7/11 patients displayed a predominant type 2 or type 3 ultrastructure. The totality of these were capable of Ig secretion, although in one case, to be discussed below (patient E), there was a discrepancy between the scarce quantity of the secretory product and the abundance of type 2 cells.

The above findings are in agreement with previous observations indicating that there is a process of maturation within the different CLL clones.\(^7\) These clones may vary as to their degree of differentiation, secretion taking place only in those clones capable of maturing to a greater extent. However, both ultrastructural and immunofluorescence studies show that secretion takes place also in the absence of plasma cells from the cell population tested. These data support the findings of Stevenson et al.,\(^32\) who detected polymeric IgM sharing the same idiotype of the neoplastic cell membrane Ig in the serum of some CLL patients, and should be taken into account when therapies employing antiidiotypic antibodies are undertaken.\(^33,35\)

Lysates of endogenously labeled cells contained a \(\mu\) chain of an apparent molecular weight of 79 ± 4 kd or a \(\gamma\) chain of an apparent molecular weight of 55 kd. These molecules were likely to represent the precursors of the secretory heavy chains, as shown by both the results of pulse-chase experiments and of those with tunicamycin. In the case of IgM, the \(\mu\) chain most likely also contained the precursor of the \(\mu\) chain, which was possibly indistinguishable from the precursor of the \(\mu\) chain in its partially glycosylated form. Support of this hypothesis would come from the obser-
vation that, following tunicamycin treatment, two \( \mu \) molecules were detected, the slower and less abundant of which probably represented the \( \mu_m \) precursor. These data are in agreement with those of Sidman in a murine experimental system.\(^5\) A precursor of \( \gamma_m \) was not detected. The finding of a \( \gamma \) chain with the same mobility as the \( \gamma_m \) molecule in the lysates of the cells harvested very early in the pulse-chase experiments suggests that this chain was a precursor molecule that could not be separated from the complete \( \gamma_m \) by SDS-polyacrylamide gel electrophoresis. These data could be explained by the scarce degree of glycosylation of \( \gamma \) chains (only one carbohydrate side chain) as compared to \( \mu \) chains (five side chains).\(^36\)

The finding of precursors of the secreted molecules in the malignant clones that failed to secrete may suggest that secretory heavy chain production begins early during B-cell differentiation. This possibility would be supported further by the “immature” morphological features of these clones detected at the ultrastructural level. Studies on a pre-B-cell line have revealed the production of both \( \mu \) and \( \mu_m \) molecules that are subsequently degraded intracellularly.\(^37\)

Therefore, the synthesis of the secretory heavy chains does not lead per se to Ig secretion, but it appears that the cells should also acquire a secretory apparatus. In the absence of such an apparatus, the secretory heavy chain precursors could be degraded intracellularly, as is suggested by the results of the pulse-chase experiments. According to this model, only a proportion of cells from the secreting malignant clone would actually secrete, namely, those cells at more mature stages, whereas the less mature cells would degrade the secretory molecule intracellularly. This would explain why some secretory heavy chain degradation was noted also in the secreting leukemias. Alternatively, some degradation of the secretory molecules could also occur in the more mature secreting cells. It is worth noting in this connection that a process of intracellular degradation accompanying the synthesis of secretory products has been described for a number of endocrine cell types and named crinophagy.\(^38,39\)

The ultrastructural studies reported here have shown that the process of B-cell maturation occurs not only through the progressive development of the synthetic and secretory machinery (i.e., RER and Golgi apparatus), but also involves the appearance of vesicles of various types, some of which could be related to the lysosomal system. It is possible that, in addition to subserving a transport function, the vesicles are engaged in the intracellular regulation (including degradation) of the secretion product. An imbalance within this regulatory mechanism would lead to defective secretion, as could have possibly been the case in patient E.

The present experiments, together with previous data,\(^37\) would indicate certain maturational steps for Ig production and secretion, namely: (a) synthesis of membrane and secretory heavy chains followed by intracellular degradation; (2) insertion of Ig molecules into the cell membrane, concomitant with the beginning of light chain production (in this case, the precursors of the secretory heavy chains would be still degraded intracellularly); and (3) secretion of Ig concomitant with the appearance of a secretory apparatus.

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