

EXTRACELLULAR IDIOTYPIC IMMUNOGLOBULIN ARISING FROM HUMAN LEUKEMIC B LYMPHOCYTES*

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Chronic lymphocytic leukemia (CLL)¹ arises usually by proliferation of a clone of B lymphocytes, and as such the cells carry some or all of the surface markers associated with B lymphocytes—notably surface Ig and a receptor for the complement component C3. Typical cells contain little intracellular Ig and are not considered to be exporting cells, except in the few cases where a proportion has evidently differentiated further to form plasma cells (1).

Previous experiments (2) have demonstrated that it is possible to raise anti-idiotypic antibody to the surface Ig of leukemic lymphocytes by treating the cells with papain and injecting the purified Fab fragments into sheep. Such antibody has a potential role in immunotherapy of these and related tumors (3) but the efficacy of such therapy clearly will be affected by the presence of idiotypic Ig in the plasma. Accordingly, the ability of the cells to export idiotypic Ig *in vitro* and to cause an accumulation in plasma *in vivo* has been investigated.

Our findings suggest that export of a small amount of pentameric IgM is a frequent occurrence in CLL, and that this is sometimes accompanied by export of IgD. In fact, we have yet to find a case of CLL in which the cells express surface Ig without simultaneously exporting it through the separate export pathway. As well as its implications for immunotherapy with anti-idiotypic, this finding suggests that idiotypic Ig in plasma might be useful in charting the progress of B lymphocytic neoplasms.

Materials and Methods

In general, the techniques applied to the cells from patients were based on those developed using a guinea pig model of a B cell proliferation, the L₂C leukemia (4).

Preparation of Fab μ from Cell Surfaces. Well-washed peripheral blood lymphocytes were suspended in phosphate-buffered saline (PBS; 0.03 M phosphate buffer that contains 0.12 M NaCl, pH 7.3) at 8×10^8 /ml and incubated with papain (0.6 mg/ml at 37°C for 60 min) to cleave the surface IgM *in situ* and to release Fab μ into the supernate (3). After a preliminary purification of the supernate by passage through DEAE-cellulose (0.06 M NaCl, 0.02 M Tris-HCl; pH 7.4) and Sephadex G-50, the Fab μ was isolated on an immunosorbent column consisting of sheep purified anti-human Fd μ coupled to Sepharose 4B-CL.

Preparation of Anti-Idiotypic Antibodies. This was carried out by a modification of our earlier method (3). The cell surface Fab μ isolated on an immunosorbent column was used as a nucleus to build up immune complexes by passing through the washed-column sheep anti-human IgM.

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¹ *Abbreviations used in this paper:* CLL, chronic lymphocytic leukemia; FITC, fluorescein isothiocyanate; MEM, minimum essential medium; PBS, phosphate-buffered saline; RIA, radioimmunoassay(s).

After further washing, Fab μ and its attached fluid-phase antibodies were eluted with 0.5 M NH₃, 1.0 M KSCN, and immediately transferred back to neutral buffer by passage through Sephadex G-25. In the amounts available for immunization (normally ~10 μ g Fab μ /animal) the immune complexes were found to be much more immunogenic than pure Fab μ . Immunization was routinely carried out in two sheep per patient: primary and booster doses of immune complexes that each contained 1–10 μ g Fab μ were given 4 wk apart and consisted of subcutaneous injections in complete Freund's adjuvant in the four shanks. The animals were bled from the jugular vein 1 wk later and the IgG fraction prepared from the serum. Antibody activity against the constant regions of the Fab μ was removed by passing the IgG down immunosorbent columns that contained coupled human IgM and coupled human serum globulins. The absorbed IgG preparations were then checked for anti-idiotypic activity specific for their homologous CLL cells.

Immunosorbents, Immunoglobulins, and Radioimmunoassays. Solid-phase immunosorbents were prepared by coupling antibody to either Sephadex G-25 Superfine or Sepharose 4B-CL (5), the former was coupled at a ratio of 25 mg IgG:5 g Sephadex and used for radioimmunoassay (RIA), the latter at 10 mg IgG:1 ml Sepharose and used for preparative purposes. RIA was applied to the following antigens: normal human pentameric IgM, normal human IgD, κ - and λ -Ig light chains, and the idiotypic Ig on the cell surfaces of three patients with CLL. In each case the radioactive antigen was labeled with ¹²⁵I to an activity of ~30 μ Ci/mg by the Iodo-gen (Pierce Chemical Co., Rockford, Ill.) method (6) which was found to be more efficient and less damaging than the chloramine T method, especially for the idiotypic antigens.

The RIA for normal human IgM used sheep anti-human Fd μ coupled to Sephadex G-25 with Fab μ as radiolabeled antigen; this assay detects pentamer, monomer, or fragments of IgM. Pentameric IgM was prepared from normal human serum by immunosorption using Sepharose 4B coupled to sheep anti-human Fd μ . Bound IgM eluted by passing a one-third-column vol of 0.5 M NH₄OH-1.0 M KSCN through the column; eluted protein was collected in ice and rapidly dialyzed into cold 0.1 M Tris-HCl-1 M NaCl-0.2% NaN₃, pH 8. Pentameric IgM was purified finally by chromatography on a column of Ultrogel ACA 22 (LKB Produkter, Bromma, Sweden).

Monomeric IgM was prepared from pentameric IgM by reduction in the presence of 2-mercaptoethanol (0.1 M) at pH 8.0 for 1 h at room temperature, followed by alkylation with iodoacetamide. It was separated from unreduced material by passage through a column of Ultrogel ACA 34. Fab μ could be prepared then by treatment with papain (0.1 mg/ml) at pH 7.4 in the presence of dithiothreitol (1 mM) for 1 h at 37°C. Reaction was stopped by addition of iodoacetamide (5 mM) and the Fab μ separated on a column of Sephadex G-100.

RIA for IgD used sheep anti-normal human Fd δ in the solid phase and radiolabeled monomeric normal human IgD as antigen. IgD was prepared from normal human serum by immunosorption. RIA for κ - and λ -light chains used sheep antibody to Fab' $\gamma\kappa$ or Fab' $\gamma\lambda$ on the solid phase and the appropriate radiolabeled light chain isolated from a suitable urinary Bence Jones protein as antigen. The assays were relatively insensitive to light chain in combination with μ - or δ -chain (Figs. 4 and 5). RIA for idiotypic Ig used sheep anti-idiotypic coupled to Sephadex G-25, with idiotypic IgM, isolated as described below, as radiolabeled antigen.

Isolation of Idiotypic IgM. Idiotypic IgM was prepared from the plasma of leukemic patients by a two-stage immunosorptive procedure as follows: first, Sepharose 4B was coupled to sheep antibody to human Fd μ to isolate all the plasma IgM; second, Sepharose 4B was coupled to sheep anti-idiotypic to separate idiotypic IgM from normal IgM. In the cases where IgD was produced in vitro, the plasma IgD was isolated by an anti-Fd δ immunosorbent, followed again by anti-idiotypic. All separations were monitored by RIA.

Ig Produced in Culture. Leukemic cells were prepared from peripheral blood by gradient centrifugation on Ficoll-Hypaque (7) and washed in PBS followed by Eagle's minimal essential medium (MEM) that contained 1% nonessential amino acids (Flow Laboratories, Inc., Walkersville, Md.), 2 mM L-glutamine, and 100 IU/ml of both penicillin and streptomycin. This medium was supplemented with 10% fetal calf serum for incubation of the cells for 2 h at 37°C to remove cytophilic Ig and adherent cells. The cells were resuspended in fresh supplemented medium and cultured at 2 \times 10⁷/ml at 37°C with gentle swirling. To assess production of Ig,

aliquots were removed at intervals and, after centrifugation to remove the cells, the supernates were analyzed by RIA.

To estimate the molecular size of the Ig produced, a sample of culture fluid obtained after incubation for 6–8 h was collected and concentrated 10 times in an Amicon ultrafiltration apparatus with a PM 10 membrane (Amicon Corp., Scientific Sys. Div., Lexington, Mass.). A 1-ml sample of the concentrate was then applied to a column of Ultrogel ACA 22 (1.6 × 77 cm) equilibrated with assay buffer that contained hemoglobin (20 µg/ml). Fractions were collected and analyzed by RIA. Markers of pentameric IgM and monomeric IgD were used to calibrate the column.

Radioiodination of Leukemic Cells. CLL cells were surface radioiodinated with ¹²⁵I by a modified lactoperoxidase method (8). A suspension of cells (0.5 ml) at 8 × 10⁶/ml in PBS was treated with 10 µl (10 µg) of lactoperoxidase (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) together with 1 mCi ¹²⁵I at 30°C. Incorporation was initiated by addition of 25 µl of 10⁻³ M H₂O₂ in PBS and additional 25-µl aliquots were added at 1, 2, and 3 min. The reaction was stopped after a total of 5 min by dilution with ice-cold PBS, and cells were washed twice with PBS.

Treatment of Cells with Papain. The treatment of cells with papain was carried out as described previously (3). To remove the majority of the Fab μ from the cell surface with minimal damage to the cells, incubation was for 30 min in the presence of papain at 0.4 mg/ml; for removal of Fab δ , incubation was for 5 min in the presence of papain at 0.02 mg/ml. After digestion, the cell suspension was cooled in ice and centrifuged. Iodoacetamide (2.6 mM) was added to the supernate to inactivate the papain, and cells were washed twice before culture.

Isolation of μ -containing Material from Papain Digests or Cell Culture Supernates. This was carried out by immunosorption described previously for the guinea pig leukemia (4), except that the immunosorbent was Sepharose 4B coupled to sheep anti-human μ -chains. After elution and dialysis, the radiolabeled product was passed through a column of Ultrogel ACA 22 (1.6 × 77 cm) to examine molecular size and homogeneity.

Plasmapheresis. Plasma exchange was carried out with a Hemonetics 30 discontinuous flow cell separator (Hemonetics Corp., Braintree, Mass.). A total of 4 liter of plasma was removed during the course of 3 h and was replaced by 2 liter of plasma protein fraction, 1 liter of blood-group-compatible fresh frozen plasma, and 1.5 liter of 0.15 M NaCl.

Immunofluorescence. To characterize the surface Ig of the CLL cells, washed lymphocytes were incubated at 37°C in supplemented MEM to remove cytophilic Ig, washed again, and then examined for reactivity with fluorescein isothiocyanate (FITC)-labeled antibodies with the following specificities for human Ig determinants: anti- μ , anti- δ , anti- γ , anti- α , anti- κ , anti- λ , and anti-Fab¹ γ (polyspecific). Intracellular Ig was examined by similar treatment of cytocentrifuged fixed cells on microscope slides.

Indirect immunofluorescence was used to examine the reactivity of anti-idiotypic antibody with cells. Leukemic cells were treated with homologous or heterologous anti-idiotypic or normal sheep IgG at 0.5 mg/ml, washed three times with PBS that contained 10 mM NaN₃, and then reacted with FITC-labeled rabbit antibody specific for sheep IgG.

Results

The surface Ig isotypes detected on the peripheral blood lymphocytes of the nine patients are shown in Table I. In all cases ~95% of the cells had the same staining characteristics; no plasma cells were detected although cells in cytocentrifuged preparations from patients Bur. and Phi. demonstrated very faint intracellular staining for Ig MK and Ig MDK respectively. None of the patients had any paraprotein detectable by conventional serum electrophoresis, nor any proteinuria.

Characterization of the Three Anti-Idiotypic Antibodies. Immunodiffusion on Ouchterlony plates showed no lines of precipitation of the anti-idiotypes with normal human serum or normal IgM pentamer over a wide range of concentrations. However, the idiotypic IgM preparations from the three patients' plasmas (Materials and Methods; and see below) formed lines of precipitation with their homologous antibodies, but

TABLE I
Surface Ig Isotypes Expressed on Peripheral Blood Lymphocytes of CLL Patients

Patient	Lymphocytes in blood $\times 10^7/ml$	Surface Ig* isotypes
Bur.	2.2	IgMG K weak
Wil.	7.0	IgMD K weak
Sim.	20	IgMD K weak
Phi.	3.1	IgMDG K
Haw.	7.0	IgMDG K
Wat.	7.9	IgMDG λ
War.	9.2	IgMDG λ
Jen.	11	IgMDG K
Han.	5.0	IgMD K weak

* In all the preparations of peripheral blood lymphocytes examined, >95% of the cells showed the surface isotypes indicated.

only at antigen concentrations ≥ 0.7 mg/ml. This paradoxical dependence of precipitation on a high antigen:antibody ratio was noted previously for the guinea pig idiotype IgM and may be a result of lattice stabilization by the pentameric antigen (4).

Immunofluorescence was used to demonstrate a strong reactivity of the leukemic lymphocytes with the homologous anti-idiotype, with $\geq 90\%$ of the cells positive. Each anti-idiotype was also tested against at least two other CLL patients' lymphocytes, which carried surface Ig of the same heavy- and light-chain class; no reaction was observed.

Specificity of the anti-idiotypes was also showed in the RIA: each was sensitive to homologous IgM idiotype in the range of 4–400 ng and showed no interference by normal pentameric IgM, normal plasma, or plasma from other CLL patients (Fig. 1). Similarly, only culture fluid obtained from the patient's lymphocytes reacted in the corresponding idiotype assay.

Production of Ig in Culture. The results of measurement of IgM levels in the culture fluids obtained from the peripheral blood lymphocytes of these patients are shown in Fig. 2. It is clear that cells from all the patients have produced IgM, although the amounts are variable, whereas a patient with CLL of T cell origin, defined by a lack of surface Ig and rosette formation with sheep erythrocytes, produced no IgM during an 8-h incubation. IgD production was seen in three patients (Fig. 3); the remaining six did not export detectable IgD even though five of the six expressed IgD at the cell surface.

The molecular size of the IgM in the culture fluid after 6–8 h of incubation was estimated by gel chromatography for the three patients examined in depth (Wat., War., and Jen.); in all three cases it was found to be predominantly pentameric (Fig. 4 shows the results for Jen.). In two of these patients who also produced IgD (Wat. and War.), the size of the major fraction of the IgD was consistent with that of monomeric IgD, although there was also a component of high molecular weight, which may be aggregated or bound to other material (Fig. 5).

The leukemic lymphocytes from all three patients also produced free light chains during culture (Figs. 4 and 5). This is a common feature of CLL cells and may relate

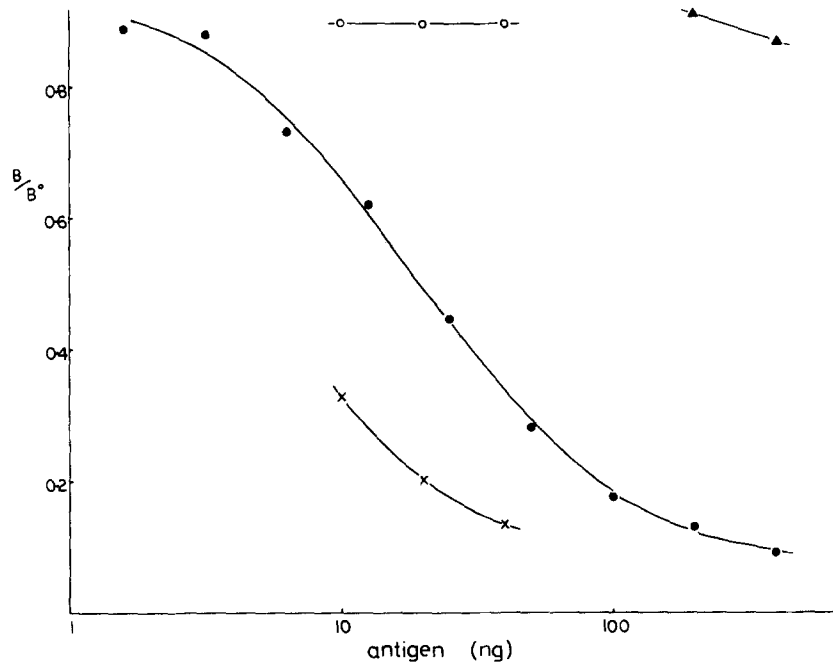


FIG. 1. Radioimmunoassay of idiotypic IgM from patient Jen. B/B° represents the fraction of total radiolabeled IgM idiotype from Jen. bound to the solid-phase anti-Jen. idiotype antibody at various concentrations of competing antigen. (●) Standard curve using Jen. IgM idiotype; (▲) normal human pentameric IgM; (×) Jen. plasma; (○) Wat. serum, (the plasma and serum preparations were diluted to 1,600:1 to give the highest concentration of antigen).

to their stage of differentiation (9). The light chains produced by the three patients did not react in the corresponding idiotype assay.

Isolation and Estimation of Idiotypic Immunoglobulins from Patients' Plasmas. Idiotypic Ig was isolated from the patients' plasmas by immunosorption as described in Materials and Methods. A flow diagram of the method for one patient (War.) is shown in Fig. 6, and the results of RIA for IgM, IgD, and idiotype in the various fractions shown in Table II. It is clear that the immunosorbents have taken up the relevant Ig and that both idiotypic IgM and IgD are present and represent (by depletion, as recovery is never 100%) ~65% and 78% of the total Ig class, respectively. One finding in the War. plasma not seen in Jen. or Wat. is that idiotype was detected in fraction A₂; i.e., neither IgM nor IgD; the nature of this material is under investigation. Results for the three patients are summarized in Table III. The depletion of normal human IgM pentamer on passage through the anti-idiotypic immunosorbents was ~15%.

Effect of Plasmapheresis on Total IgM and Idiotype in Serum. Plasmapheresis was carried out on two patients and the results of this procedure on subsequent levels of IgM and idiotype are summarized in Table IV. Levels of IgM postplasmapheresis reflect the fact that fresh-frozen plasma was infused into the patient to replace fluid loss; this has caused an increase in the normal IgM levels but has not affected the fall in idiotype levels. In the patient War., the idiotype levels reflect contribution from idiotypic IgM and IgD. The results on patient Jen. are not complicated by this as only idiotypic

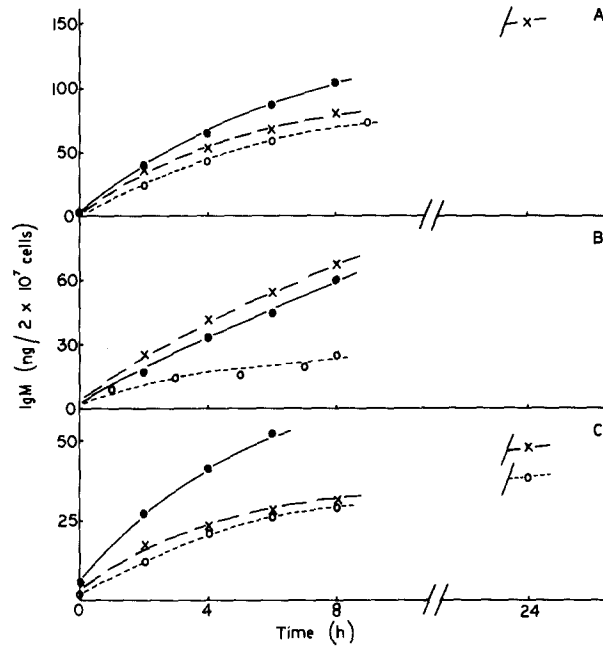


FIG. 2. The production of extracellular IgM by CLL cells in culture. Peripheral blood lymphocytes from nine patients were cultured, and samples of centrifuged culture medium examined for IgM by RIA at the times indicated. Cell viabilities by trypan blue exclusion were >95% up to 10 h of culture, and decreased to 80-85% at 24 h. (A) (●) Haw.; (×) Wat.; (○) Jen. (B) (●) War.; (×) Han.; (○) Bur. (C) (●) Phi.; (×) Wil.; (○) Sim.

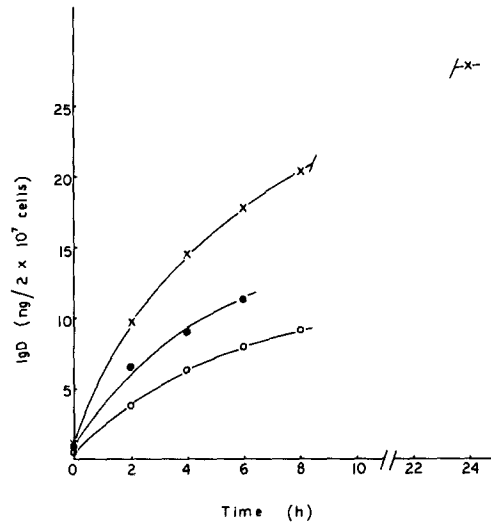


FIG. 3. The production of extracellular IgD by CLL cells in culture. Peripheral blood lymphocytes from nine patients were cultured, and samples of centrifuged culture medium examined for IgD by RIA at the times indicated. Only three cultures produced IgD: (●) Phi.; (×) Wat.; (○) War.

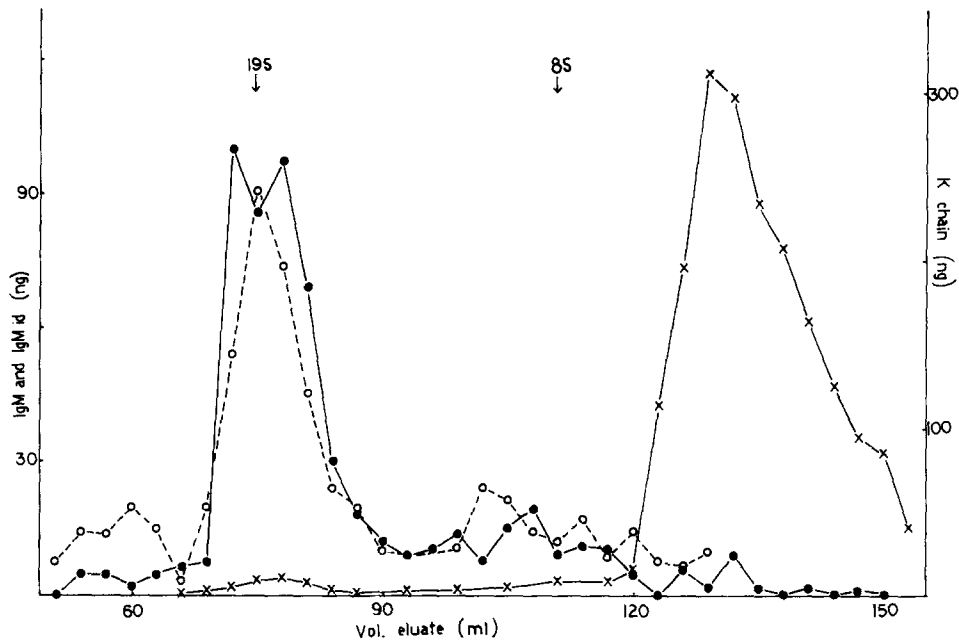


FIG. 4. Separation of culture fluid from Jen. cells by chromatography on Ultrogel ACA 22. Cells were cultured at 2×10^7 cells/ml for 7 h, the culture fluid collected by centrifugation, and concentrated 10 times by ultrafiltration. 1 ml was then passed through an Ultrogel ACA 22 column (1.6×77 cm) and fractions examined for IgM, idiotype, and κ -chains by RIA. Recoveries of these components from the column were in the range of 75–85%. (○) IgM; (●) idiotype; (×) κ -chains.

IgM was present in this patient; there is a small decrease in total IgM and a considerable fall in idiotypic IgM.

Investigation of the Origin of Idiotypic Ig. It was of interest to establish whether the idiotypic Ig found in culture fluids or sera of patients arose by secretion or by turnover from the cell surface. This question was approached in the two ways previously described for the guinea pig leukemic cells (4). First, cells from patient Jen. were surface labeled with ^{125}I by the lactoperoxidase method and papain was used to split off the labeled $\text{Fab}\mu$ (3). This was collected by immunosorption and separated on a column of Ultrogel ACA 22. Results are shown in Fig. 7 where the two peaks indicated represent $\text{Fab}\mu$ fragments of different sizes arising from the papain cleavage of surface IgM (A. Abdul-Ahad and G. T. Stevenson. Personal communication.). Thus the $\text{Fab}\mu$ -associated radioactivity released by the papain gave rise to $\sim 1,500$ cpm of ^{125}I . In a parallel experiment with nonradioactive ^{127}I in place of ^{125}I so that RIA may be applied, the actual amount of $\text{Fab}\mu$ released by papain was found to be 5 pmol; this amount therefore corresponded to 1,500 cpm from the radiolabeled cells.

An aliquot of radiolabeled cells that had not been treated with papain was then placed in culture for 8 h, after which the released IgM was collected by immunosorption. Chromatography on Ultrogel ACA 22 showed no labeled IgM in the pentameric or monomeric positions; only high molecular weight (possibly vesicle-bound) material was detected together with some material in the $\text{Fab}\mu$ position. However, RIA of the parallel nonradioactive ^{127}I -labeled cell culture showed production of 18 pmol of mainly pentameric IgM during the 8-h period, none of which apparently had been

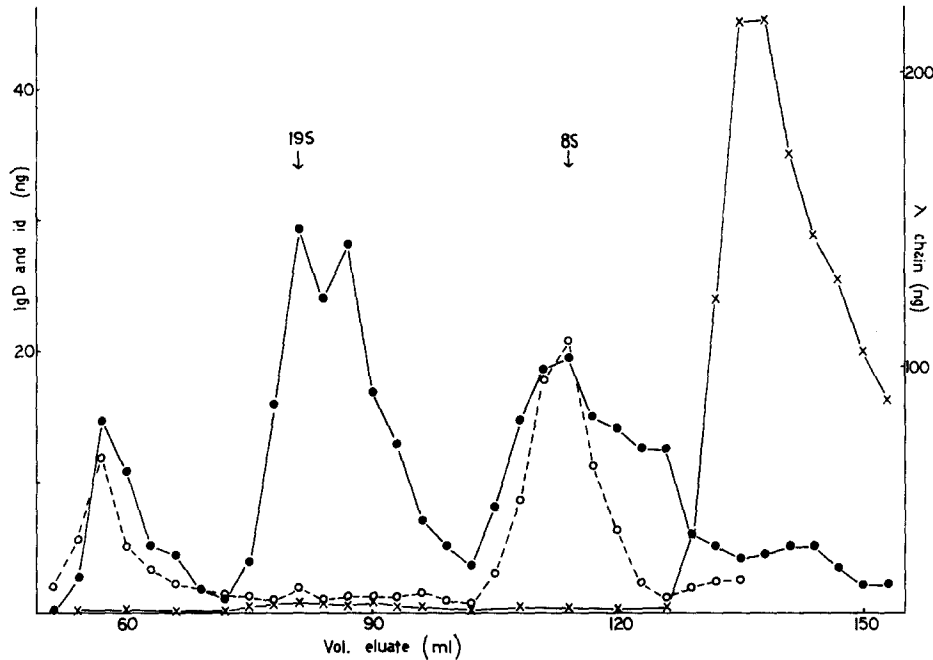


FIG. 5. Separation of culture fluid from War. cells by chromatography on Ultrogel ACA 22. Cells were cultured at 2×10^7 /ml for 6 h, the culture fluid collected by centrifugation and concentrated 10 times by ultrafiltration. 1 ml was then passed through an Ultrogel ACA 22 column (1.6×77 cm) and fractions examined for IgM, IgD, idiotype, and λ -chains by RIA. Recoveries of these components from the column were 75–85%. The IgM results have been omitted for clarity, but showed a peak at the 19S position coincident with idiotype. (○) IgD; (●) idiotype; (×) λ -chains.

available for labeling by lactoperoxidase. This suggests that this IgM is produced in culture by an export pathway.

A second approach was used to examine the origin of IgD. Lymphocytes from patient War. were treated for 5 min with papain at 0.02 mg/ml to remove the Fab δ from the cell surface; removal was complete as judged by immunofluorescence. Enzyme-treated and control cells were then cultured and the fluids examined by RIA for released IgD; the assay was specific for the Fd δ region of IgD. Results are shown in Fig. 8 and demonstrate that production of IgD was hardly affected by the treatment with papain and that surface IgD therefore must contribute little to the material in the culture fluid.

Discussion

The disease CLL, usually seen as a B lymphocyte proliferation, is evidently heterogeneous in cell type. The majority of patients appear to have leukemic lymphocytes that bear IgM and IgD on their surfaces, both of which carry the same idiotypic determinants (10), but contain very little intracellular Ig. However, in a few cases, a low percentage of plasma cells is present and these can be associated with a monoclonal Ig in the serum (1). There is also a report (11) that suggests that highly refined immunoelectrophoresis can detect monoclonal Ig in association with the disease more frequently.

In the typical nonexporting CLL, it has proved difficult to study distribution and

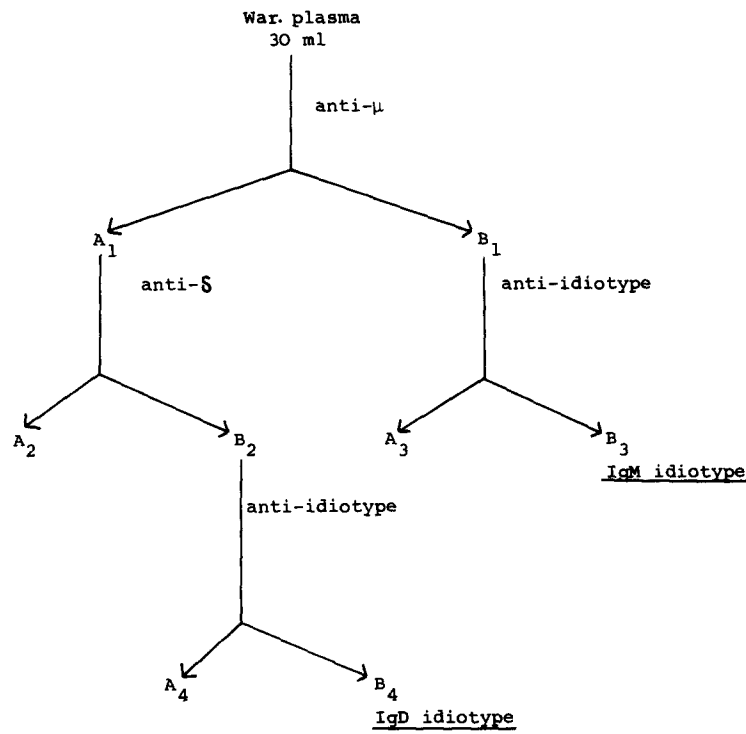


FIG. 6. Separation of War. plasma on immunosorbents to isolate idiotypic IgM and IgD. The A fractions are those unretarded by immunosorbent, the B fractions those eluted by 0.5 NH_4OH -1.0 M KSCN. RIA results on the fractions are shown in Table II.

TABLE II
Fractionation of Plasma from Patient War. to Isolate Idiotypic Immunoglobulins

Fraction*	IgM	IgD	Idiotype
		$\mu\text{g}/\text{fraction}$	
Plasma	960	327	1830
A ₁	3.6	406	653
B ₁	662	0.6	272
A ₂	—	0.2	262
B ₂	—	175	74
A ₃	233	—	0.35
B ₃	319	—	343‡
A ₄	—	38	0.52
B ₄	—	104	134§

* Fractions are as indicated in Fig. 6.

‡ IgM idiotype.

§ IgD idiotype.

dynamics of the tumor because of a lack of anti-idiotypic. However, the method developed using the guinea pig B lymphocytic leukemia for raising anti-idiotypic antibody against papain digests of leukemic cell surfaces has proved applicable to human CLL, even though the latter cells carry a much lower density of surface Ig.

TABLE III
Levels of Normal and Idiotypic IgM and IgD in the Plasma of Three CLL Patients*

Patient	Total plasma IgM	Idiotypic IgM (percentage of total)‡	Total plasma IgD	Idiotypic IgD (percentage of total)‡
	$\mu\text{g/ml}$		$\mu\text{g/ml}$	
Wat.	109	57	17	59
War.	39	65	11	78
Jen.	579	93	1.9	—

* All estimations of immunoglobulins were by RIA.

‡ The percentage of idiotypic Ig was calculated by depletion of Ig on an immunosorbent specific for that idiootype.

TABLE IV
Effect of Plasmapheresis on Levels of Total IgM and Idiotypic Ig in Two CLL Patients*

Patient	Total IgM‡			Idiotype§		
	Prepheresis	Postpheresis	Percent change	Prepheresis	Postpheresis	Percent change
	$\mu\text{g/ml}$			$\mu\text{g/ml}$		
War.	100	240	+140	339	174	-49
Jen.	579	429	-26	403	156	-61

* All estimations were by RIA.

‡ Total IgM in the plasma is the net result of removal by plasmapheresis and infusion of fresh frozen plasma.

§ Reactivity is expressed in terms of equivalents of pentameric IgM, the standard for the assay. In the case of War., this reactivity includes a contribution from idiotypic IgD.

Anti-idiotypic antibody has been raised against cell surface Ig of three patients and has been used to assess the ability of the leukemic lymphocytes to export idiotypic Ig. It has been found that the cells from these patients all export pentameric idiotypic IgM in small quantities (160–480 molecules of pentamer/cell per h); such export may be a common property of this cell type as a further six out of six patients also exported pentameric IgM; although in these cases the idiotypic nature has not yet been demonstrated.

It was also found that three out of nine patients' lymphocytes exported monomeric IgD (230–460 molecules of monomer/cell per h), again established as idiotypic for patients War. and Wat. where antibody was available. It is evident that not all CLL lymphocytes that carry surface IgD engage in detectable export.

The origin of the pentameric IgM and monomeric IgD appears to be via an export pathway without a surface phase rather than by the shedding of surface material, again showing close analogy with the guinea pig leukemia (4). However, if cells are stressed during culture, it is possible that surface membrane will be shed as vesicles, a phenomenon that has been reported previously (12) and that may account for the high molecular weight material found after lactoperoxidase-catalyzed iodination of the cell surface. As discussed previously (4) such vesicle-bound Ig cannot be readily distinguished from soluble Ig by the technique of immune precipitation followed by dissolution in sodium dodecyl sulfate before electrophoresis. The fate of Ig discarded as a consequence of normal turnover at the cell surface is not clear: shedding in vesicles or endocytosis and degradation are both possibilities. The latter process could

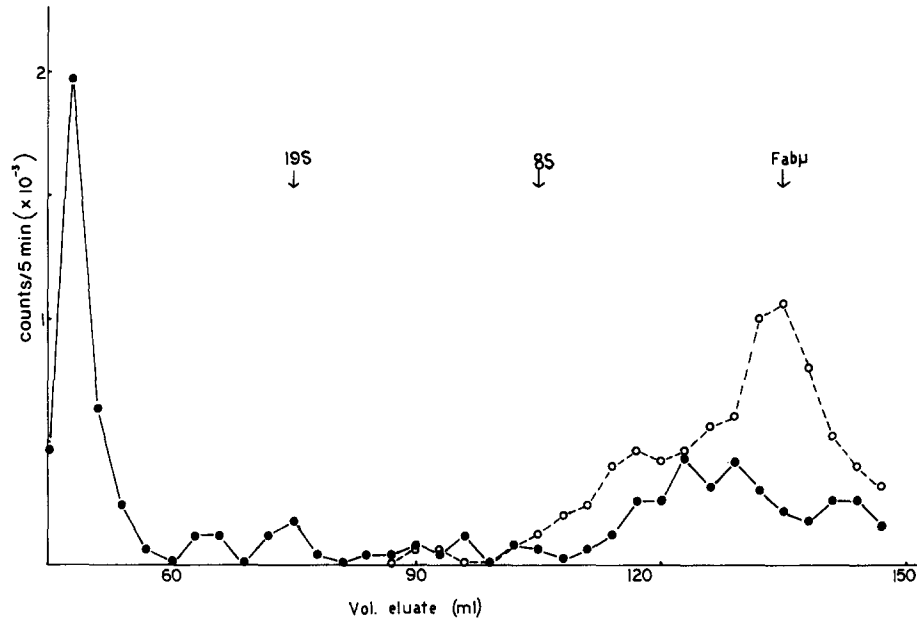


FIG. 7. Investigation of products of turnover of IgM on the cell surface of Jen. cells. Cells from Jen. were radiolabeled on the surface with ^{125}I by lactoperoxidase and an aliquot treated with papain; the μ -related released product was isolated by immunosorption and chromatographed on Ultrogel ACA 22: (○) μ -related material from the papain digest. A further similar aliquot of radiolabeled cells not treated with papain was cultured for 8 h, and the μ -related released product isolated by immunosorption and chromatographed on Ultrogel ACA 22: (●) μ -related material from culture fluid.

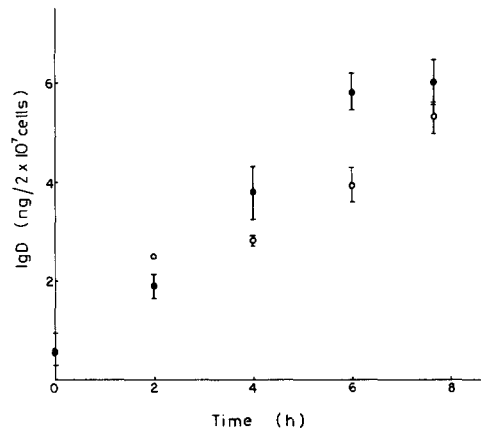


FIG. 8. Investigation of the source of extracellular IgD produced by War. cells. An aliquot of War. cells was treated briefly with papain to remove surface $\text{Fab}\delta$, washed, and placed in culture with an aliquot of untreated cells. IgD released into the culture fluids was estimated in duplicate samples by RIA specific for $\text{Fab}\delta$. (○) Papain-treated cells; (●) untreated cells.

account for the production of the $\text{Fab}\mu$ -related material in the culture medium of the lactoperoxidase-labeled cells.

It should be noted that cells from the three patients examined in detail all also exported monotypic Ig light chains during culture. This phenomenon has been

described recently (9), and it has been shown that a proportion of patients with CLL can export sufficient light chains to be detected by sensitive methods in the urine (13). In all three cases here it was found that the light chains did not react in the idiotype assay, suggesting that the antibody recognizes either heavy-chain determinants only or those that depend on heavy-light combinations (14).

The behavior of the CLL cells in culture appears to be reflected in the plasma of the patients investigated. Thus, patients War. and Wat. each had idiotypic IgM and IgD in the plasma, whereas Jen. had idiotypic IgM only. The proportion of idiotypic Ig found in each Ig class may reflect the course of the disease and measurement of changing levels could possibly be used to monitor therapy. Plasmapheresis can reduce the load of idiotypic Ig in the plasma and may be useful before immunotherapy by passive anti-idiotypic antibody.

Summary

The peripheral blood lymphocytes of nine out of nine patients with typical surface Ig-positive chronic lymphocytic leukemia but no paraprotein visible on serum electrophoresis have been shown by radioimmunoassay to export small amounts of pentameric IgM during culture (in the range of 2.4–7.2 ng/10⁷ cells per h); three out of nine also exported monomeric IgD (0.7–1.4 ng/10⁷ cells per h). Immunoglobulin turned over on the cell surface did not appear to contribute to material in the culture fluid, except possibly as vesicle-bound Ig. In three cases, which included two of the IgD producers, anti-idiotypic antibody raised against the cell surface Fab μ was used to demonstrate the idiotypic nature of the exported Ig.

Anti-idiotypic antibody was also used to measure levels of idiotypic Ig in the sera of these three patients as a proportion of the total Ig. Total serum IgM was depressed in all three patients, and the idiotypic IgM represented 43%, 65%, and 96% of the IgM.

The findings suggest that in typical chronic lymphocytic leukemia involving B lymphocytes, the export of a small amount of idiotypic Ig by the neoplastic cells is a common or even usual occurrence.

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