

IDIOTYPIC UNIFORMITY OF CELL SURFACE IMMUNOGLOBULIN IN CHRONIC LYMPHOCYTIC LEUKEMIA

Evidence for Monoclonal Proliferation*

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Chronic lymphocytic leukemia (CLL) is thought to be a malignancy of bone marrow-derived (B) lymphocytes, since the leukemic cells from a majority of patients bear easily detectable membrane-bound immunoglobulin (Ig) (1), possess a complement (C3) receptor (2), and do not bind sheep erythrocytes (3). Since in most studies (1, 3-5), but not all (6), the cells from an individual patient bear Ig restricted to a single light chain class, it is suspected that the CLL lymphocytes represent the progeny of a single clone of B cells.

The present study provides support for the clonal proliferation of CLL cells by demonstrating in a single patient that the Ig on virtually all of the CLL cells possess the same idiotypic (variable region) determinants. The anti-idiotypic serum, prepared against a circulating paraprotein, reacted only with the paraprotein and the surface Ig on the CLL cells and not with a variety of other serum or cell-bound Igs.

Materials and Methods

Source and Isolation of Lymphocytes. J. T. is a 61-yr old male with an 18 mo history of chronic lymphocytic leukemia and an IgG paraprotein. The peripheral lymphocyte count has been maintained at $\leq 20,000$ mature lymphocytes per mm^3 by two, brief courses of chemotherapy. The IgG paraprotein has persisted at 50-60 mg/ml throughout his course.

Lymphocytes were collected from peripheral blood by the methods of Böyum (7). CLL lymphocytes from 23 different patients were either used fresh or frozen viably (8) and stored at -82°C for periods of up to 9 mo before use. Viability of the thawed cells ranged from 40-82%. Preliminary experiments demonstrated that viably frozen cells maintained their surface immunoglobulin. Lymphocytes (PBL) from 16 nonhematologic patients were used fresh.

Purification and Characterization of the Paraprotein and Preparation of Anti-Idiotypic Serum. The paraprotein was purified by DEAE chromatography and preparative isoelectric focusing. It was shown by double diffusion to possess kappa light chains; Dr. R. Wistar of the Naval Medical Research Institute, Bethesda, identified its subclass as IgG₁. Heavy and light chains were separated

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after reduction and alkylation; Fab fragments were prepared by papain digestion (9). Isolated chains were recombined by the method of Bridges and Little (10) and recombinant molecules isolated on Sephadex G-150 in phosphate-buffered saline.

Anti-idiotypic serum was prepared in rabbits immunized with 1 mg of purified J. T. IgG₁ (K) emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) and administered intraperitoneally. After 6 wk, the serum was collected and passed over an immunoabsorbent column of human γ -globulin (HGG; CalBiochem, La Jolla, Calif.) coupled to Sepharose 4B (Sigma Chemical Co., St. Louis, Mo.) by cyanogen bromide. The specificity of the absorbed antiserum and the distribution of idiotypic determinants were determined by hemagglutination inhibition as described in Table I.

Preparation of Class-Specific Antisera and Fluorescent Conjugates. Class-specific, purified antibodies were prepared by adsorption and acid elution from purified myeloma proteins conjugated to Sepharose. Goat antibody to IgD, sheep antibody to kappa and lambda light chains, IgE, and the Fab' fraction of IgG, and rabbit antibodies to IgM and IgA were prepared in this way. Certain of these antisera were kindly provided by Dr. H. Metzgar, NIH. In addition, antisera to IgG, IgA, IgM, and IgD were prepared as described previously using insoluble immunoabsorbents (11, 12). The antisera and antibodies were conjugated with fluorescein (F) or tetramethylrhodamine (Rh) isothiocyanate (13).

Immunofluorescent Labeling of Cells. Lymphocytes were stained with Rh- or F-labeled antibodies or globulin fractions as previously described (11). Briefly, $1-3 \times 10^6$ cells were incubated at 4°C for 30 min in 0.1 ml minimum essential medium (Grand Island Biological Co., Rockville, Md.) containing 50 μ g/ml of Rh- or F-labeled antibodies in 10% fetal calf serum. The cells were washed twice through fetal calf serum and suspended in medium containing 10 μ g/ml of ethidium bromide for 5 min at room temperature. After a final wash the cells were examined with a fluorescent microscope equipped for vertical illumination. Dead cells are stained homogeneously red with ethidium bromide and can be easily identified under these conditions.

Results

The specificity of the anti-idiotypic serum is shown in Table I. It is clear that a number of IgG myeloma proteins and normal Ig lack the idiotypic determinant(s) that J. T. IgG paraprotein possesses. In addition, the idiotypic determinants on J. T. IgG are located on the Fab portion of the paraprotein, but not on either

TABLE I
Specificity of Anti-Idiotypic Serum

Inhibitor	HI*
	μ g/ml
J. T. IgG(K)	0.18 (0.05-0.65)‡
Fab	0.14
Heavy chain	75
Light chain	25
Recombined heavy-light chain	0.86
IgG myelomas§	> 10,000
Normal human serum	> 10,000
Normal pooled Ig	> 10,000

* Amount of protein which inhibits hemagglutination of J. T. IgG-coated sheep erythrocytes by a limiting dilution (1/300) of anti-idiotypic antiserum.

‡ Geometric mean and SE of four determinations.

§ Six separate myeloma proteins.

heavy or light chains alone. The unique specificity of the anti-idiotypic serum is supported by the fact that recombination of the heavy and light chains restores the idiotypic determinants nearly to that of the native molecule.

The J. T. CLL cells were shown to possess the same antigenic determinants that the paraprotein possessed including the idiotype (Table II). Virtually all cells possess a single heavy and light chain with a uniform idiotype. A variety of lymphocytes from normal donors and from other CLL patients all lacked the idiotypic determinants. The small fraction (0.3%) of δ -bearing lymphocytes in J. T. cell preparations were shown to lack the idiotypic determinants by double-label immunofluorescence using Rh-anti- δ and Fl-anti-idiotypic and presumably represent normal lymphocytes. Several controls indicated that the CLL cells produced the surface immunoglobulin rather than acquiring it passively from the serum. First, several control CLL suspensions were incubated at 37°C for 12–18 h in J. T. serum and washed; no significant amounts of paraprotein were adsorbed,

TABLE II
Antigenic Characteristics of J. T. CLL Surface Immunoglobulin

Reagent	J. T. CLL	PBL	CLL
Fl-anti-idiotypic	91.4	<1 (16)*	<1 (23)*
Fl-anti- γ	92.4	11.3 (6)	ND
Rh-anti- δ	0.3	3.0	ND
Fl-anti- α	<0.4	6.0	ND
Rh-anti- μ	<0.4	4.6	ND
Fl-anti- ϵ	<0.4	ND	ND
Fl-anti- κ	81.4	18.3	ND
Fl-anti- λ	<0.2	7.8	ND

* No. of individuals tested.

as determined by Fl-anti-idiotypic staining. Second, culture of trypsinized (0.3 mg trypsin/ml, 37°C, 1 h) J. T. CLL cells for 6 h at 37°C resulted in an increase in idiotype-bearing cells from 18% to 66% of viable cells.

Discussion

There is now considerable evidence for the monoclonal nature of the cellular proliferation in chronic lymphocytic leukemia (1). In most studies, the surface immunoglobulin possesses a single light-chain type (1, 4, 5). Other instances of restriction have been reported with regard to IgG subclasses and Gm allotypes (3). However, in all instances evidence of restriction has relied on markers in the constant regions of Ig. In this communication we provide evidence for clonal proliferation by demonstrating that all CLL cells of a single patient possess identical idiotypic (variable region) markers. The approach we used was similar to that of Kunkel and colleagues who showed that a substantial fraction of the lymphocytes of two individuals with apparently idiopathic IgM paraproteinemia possessed idiotypic determinants in common with their paraproteins (14).

While most studies have demonstrated that CLL cells possess Ig restricted to a

single heavy-chain class, two recent studies have shown that a large fraction of patients with CLL possess cells bearing IgD in addition to IgM determinants (15, 16). While J. T. CLL cells under consideration in this study possessed only a single class of Ig, the use of anti-idiotypic sera should be especially valuable in the study of individual cells bearing Ig of more than one class as a means of proving variable region identity among the immunoglobulin classes. In summary, we have shown that the surface Ig on CLL lymphocytes of an individual patient possess common variable region markers, a finding which supports the thesis that the lymphoproliferation in CLL is monoclonal.

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