PRODUCTION OF AUTOANTIBODIES BY CD5-EXPRESSING B LYMPHOCYTES FROM PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Based primarily on studies in the mouse, it has been postulated that at least two separate lineages of B cells exist (reviewed in reference 1). The first lineage, characterized by the cell surface expression of the Ly-1 antigen, appears to be primarily responsible for the secretion of antibodies that display extensive autoreactivity. The second lineage, characterized by the lack of Ly-1 expression, is primarily responsible for the production of "conventional", non-self-reactive antibodies (2–5). This distinction has been most convincingly demonstrated in inbred strains of mice with a genetic predisposition for the development of various autoimmune syndromes (6, 7).

The human counterpart of the Ly-1 antigen, recently termed CD5, was originally defined using the anti-Leu-1 mAb (8). In this (8) and subsequent (9–12) studies, it was demonstrated that the expanded monoclonal population of B cells that develops in patients with chronic lymphocytic leukemia (CLL)[1] expresses the CD5 molecule. Thus, CLL appears to represent a clonal overexpansion of the putative autoantibody-producing B lymphocyte. In support of this notion are the clinical observations that the sera of patients with CLL occasionally contain autoantibodies and that ~20% of such patients develop autoimmune phenomena such as hemolytic anemia (13).

On the basis of these findings, the following prospective studies were performed to determine, at the clonal level, if the CD5-expressing B cells in patients with CLL and other $CD5^+$ B lymphoproliferative disorders secrete mAbs reactive with autoantigens. These in vitro studies demonstrate that leukemic cells from >50% of patients with overexpansions of $CD5^+$ cells synthesize and can be made to secrete Ig that is autoreactive. Considering that these secreted mAbs were screened for reactivity with only a limited panel of autoantigens, it is conceivable that an even higher

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[1] *Abbreviations used in this paper:* CLL, chronic lymphocytic leukemia; DWDL, diffuse well-differentiated lymphocytic lymphoma; EIA, enzyme immunoassay; RF, rheumatoid factor; RGG, rabbit gamma globulin; SAC, *Staphylococcus aureus* Cowan strain I.

percentage of B CLL clones may be programmed for self reactivity. These studies provide direct evidence for the secretion of autoantibodies by monoclonal populations of CD5-expressing cells and also confirm a link between CD5+ leukemias and autoimmune disease in general.

Materials and Methods

Subjects. Sterile heparinized venous blood was obtained from 19 patients with CLL, and one with diffuse well-differentiated lymphocytic lymphoma (DWDL) followed at the North Shore University Hospital, Manhasset, NY. 17 males and three females, aged 50–86 yr, were included in this group. Healthy volunteers, sex matched and age approximated, served as controls. Studies were approved by the Institutional Review Board of the North Shore University Hospital.

Cell Preparation. PBMC were fractionated into T cell- and non-T cell-enriched subpopulations by rosetting with neuraminidase-treated sheep erythrocytes as described (14). The non-T cell populations routinely contained <5% $CD3^+$ cells by indirect immunofluorescent analyses. T cells, obtained from normal volunteers, were exposed to irradiation (1,500 rad) from a cesium source and were used to provide normal allogeneic helper function (15).

Reagents. PWM (Gibco Laboratories, Grand Island, NY), PMA (Sigma Chemical Co., St. Louis, MO), and *Staphylococcus aureus* Cowan strain I (SAC; Calbiochem-Behring Corp., La Jolla, CA) were used at 1:500, 10 ng/ml, and 0.01% final concentrations, respectively, to induce B cell differentiation.

Culture Conditions. All cell suspensions were cultured in RPMI 1640 medium (Flow Laboratories, McLean, VA) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), 2 mM glutamine, and 10% heat-inactivated FCS (all from Gibco Laboratories). Cell cultures were seeded in 24-well plates (Linbro; Flow Laboratories) at 10^6 cells/ml in 1 ml vol and incubated at 37°C in a 5% CO_2 humidified atmosphere. When indicated, irradiated allogeneic T cells (3×10^6) and non-T cells (10^6) were reconstituted at a 3:1 ratio in 2 ml total vol.

Assessment of B Cell Differentiation. Non-T cells were cultured in the presence of medium alone, or appropriate amounts of PMA, SAC, or irradiated normal allogeneic T cells and PWM. Cultures were harvested on day 7, and supernatants were frozen at −20°C. The total amount of Ig in the supernatants was detected by enzyme immunoassay (EIA) as previously described (16). When indicated, assays were developed with L chain-specific (κ or λ) peroxidase-conjugated goat anti-human antibodies to determine heterogeneity of antibody populations.

Immunofluorescent Analyses. Surface membrane phenotypes were determined by indirect immunofluorescence using a battery of murine mAb and flow cytometry as described (17). In certain cases, non-T cells were sorted into enriched $CD5^+$ populations using a FACS 440 flow cytometer (Becton Dickinson & Co., Mountain View, CA) as described (18).

Assays of Anti-DNA and Rheumatoid Factor Antibodies. Levels of ssDNA and dsDNA antibodies in serum and culture supernatants were determined by solid-phase EIA according to the method of Eaton et al. (19). To permit the convenient detection of anti-gamma globulin activity by a similar solid-phase EIA, without requiring isolation of the secreted Ig from culture supernatants, plates were coated with 10 μg/ml of rabbit gamma globulin (RGG). Thus, the operational definition of rheumatoid factor (RF) in this study was binding to heterologous IgG, a technique that detects the majority, but not all, human RFs (20–23).

To allow for comparison of data obtained over the 18-mo period of this prospective study, an arbitrary binding unit system was devised in which the OD units of the experimental groups were divided by the OD units, obtained in the same assay, of monoclonal human autoantibodies reactive with RGG (Q39), ssDNA (W8; reference 18), and dsDNA (W4; reference 18), and then multiplied by 10. Values >1.0 were considered significant.

Results

Patient Population. 20 Caucasian patients (17 males and three females, aged 50–86 yr) with documented $CD5^+$ chronic B cell malignancies were studied. 19 patients

had CLL and one (No. 20) had DWDL. Table I summarizes the clinical features of these patients with current white blood cell counts, clinical stage according to the Rai classification (24), and modes of treatment. 50% of the patients showed low levels of serum gamma globulins and one patient (No. 8) had a prior episode of autoimmune hemolytic anemia; none of the others had autoimmune phenomena nor rheumatic features.

All the B cell malignancies studied were of the CD5-expressing variety. Although the percentage of $CD5^+$ cells agreed very closely with the numbers of sIg^+ and $B1^+$ cells, the density of CD5 expression varied between different patients (data not shown). In two cases, sIg was not detected by indirect immunofluorescent analyses despite repeated surface marker studies, although other B cell markers, such as DR, B1, and B4, were easily detectable. In one of these patients, IgG κ secretion was detected after in vitro culture (No. 19), whereas in the other (No. 3), no Ig synthesis was ever found. The majority of patients' B cells expressed surface IgM; 12 in association with κ L chains and three with λ L chains. Three patients displayed an IgG surface phenotype; two (Nos. 5 and 10) in association with λ L chains and the other (No. 13) in association with κ L chains.

In Vitro Ig Production by B Lymphocytes from Patients with Chronic Lymphoid Malignancies. Peripheral blood non-T cells from these patients were cultured in medium alone or

TABLE I

Clinical and Cellular Characteristics of Patients with $CD5^+$ B Cell Leukemias

Patient No.	Age	Sex	Dx	Duration of disease	Clinical stage*	WBC count ($\times 10^3$)	Surface Ig	Past/present therapy‡
	yr			*yr*				
1	65	M	CLL	4	III	40.0	μκ	chlr; pred
2	65	M	CLL	3	0	27.6	μκ	None
3	79	M	CLL	9	IV	11.5	bld§	ctx; vcr; pred
4	65	M	CLL	3	0	50.0	μκ	None
5	67	M	CLL	3	II	7.9	γλ	ctx; vcr; dex
6	70	F	CLL	9	II	87.0	μκ	None
7	67	M	CLL	1	0	32.0	μκ	None
8	68	M	CLL	4	IV	58.0	μκ	ctx; vcr; pred
9	50	M	CLL	3	IV	18.9	μλ	chlr; splenectomy
10	68	M	CLL	5	II	53.0	γλ	chlr
11	66	M	CLL	5	I	84.2	μκ	None
12	66	M	CLL	2	II	170.0	μλ	chlr; pred
13	54	F	CLL	2	II	6.0	γκ	spln irrad; ctx; vcr
14	64	M	CLL	2	IV	29.5	μκ	ctx; pred; vcr
15	59	M	CLL	>10	IV	127.0	μκ	Fludarabine
16	60	M	CLL	8	IV	57.0	μκ	ctx; vcr; pred; chlr; i.v. gamma globulin
17	86	F	CLL	>10	0	31.0	μκ	None
18	68	M	CLL	6	IV	12.5	μκ	None
19	70	M	CLL	10	II	106.0	bld	chlr; vcr; pred
20	59	M	DWDL	1	I	10.2	μλ	None

* Clinical staging according to Rai classification (24).

‡ chlr, chlorambucil; ctx, cytoxan; pred, prednisone; vcr, vincristine; dex, dexamethasone; spln irrad, splenic irradiation.

§ bld, below level of detection.

in the presence of either PMA, SAC, or irradiated normal T lymphocytes plus PWM. After 7 d, the supernatants of the individual cultures were collected and assayed for Ig content by EIA. Table II lists the results of these analyses.

Significant levels of Ig were detected in cultures from 17 of the 20 patients studied; these levels ranged from 5 to 100 ng/ml. Only three patients (Nos. 1–3) failed to produce significant amounts of Ig even after mitogenic stimulation. Several patterns of Ig synthesis were seen in the cultures of the other 17 non-T cell preparations. In six cases, stimulation by one or several mitogens resulted in the elaboration of significantly more Ig than found in unstimulated control cultures (Nos. 4–9); in six other cultures, significant, albeit less impressive, augmentation of Ig synthesis was found after mitogen stimulation (Nos. 10–15). Of these 12 patients, three were induced to differentiate with PMA (Nos. 11, 13, and 15), four with both PMA and allogeneic T cells plus PWM (Nos. 4, 6, 8, and 14), one with SAC and PMA (No. 9), three with all combinations (Nos. 5, 7, and 12), and one only by allogeneic T cells plus PWM stimulation (No. 10). In control cultures, irradiated allogeneic normal T cells failed to produce significant amounts of Ig. In cultures from patients Nos. 16–20, mitogen stimulation was either ineffective in augmenting Ig synthesis or resulted in lower levels of Ig production. Thus, heterogeneous patterns of Ig production were seen among these 20 patients with CLL and DWDL. Nevertheless, the B cell populations from the majority of patients elaborated sufficient amounts of Ig, under one or several culture conditions, to allow analysis of autoantibody reactivity.

TABLE II

Differentiation Capacities of CD5+ B Cell Malignancies

Patient No.	Disease	Cultures stimulated with: Medium	SAC	PMA	Irradiated normal T cells plus PWM
1	CLL	0.003	0.008	0.000	0.027
2	CLL	0.000	0.000	0.000	0.000
3	CLL	0.000	0.000	0.000	0.010
4	CLL	0.175	0.180	0.447	0.416
5	CLL	0.081	0.234	0.309	0.315
6	CLL	0.290	0.352	0.699	0.408
7	CLL	0.044	0.275	1.286	0.645
8	CLL	0.563	0.556	1.292	1.406
9	CLL	0.114	0.394	0.408	ND
10	CLL	0.006	0.024	0.016	0.164
11	CLL	0.104	0.111	0.260	0.104
12	CLL	0.179	0.322	0.304	0.279
13	CLL	0.416	0.496	0.780	ND
14	CLL	0.184	ND	0.572	0.429
15	CLL	0.292	0.222	0.418	0.320
16	CLL	0.526	0.470	0.722	0.596
17	CLL	0.802	0.502	0.124	0.498
18	CLL	0.624	0.707	0.326	0.332
19	CLL	0.379	0.382	0.397	0.442
20	WDL	0.808	1.093	0.953	0.711

Values represent arithmetic means of triplicate or quadruplicate determinations of OD units at 490 nm. Background values (never in excess of 0.005) have been subtracted in each case. Culture supernatants were measured at 1:5 dilution.

Autoantibody Production by CD5⁺ CLL and DWDL B Lymphocytes. Supernatants from the cultures of the 17 patients that contained significant Ig were assayed by EIA for reactivity with RGG, ssDNA, or dsDNA. As noted in Table III, Ig from cultures from nine of the 17 patients tested were found to bind significantly as RFs or anti-DNA antibodies. Although data for only one culture are reported in the table, it should be noted that every culture supernatant containing Ig was tested and found to have similar autoantibody reactivity. Up to 10 μg/ml of control monoclonal Ig purified from the sera of patients with macroglobulinemia and myeloma (which represents as much as a 100-fold excess above the Ig levels found in the most efficient leukemic cell cultures) showed no reactivity with RGG, ssDNA, or dsDNA.

Several patterns of Ig reactivity with the three autoantigens tested were seen. In certain situations, restricted reactivity with only one autoantigen was seen. For instance, the secreted Ig from patients Nos. 7 and 8 appeared to react only with RGG and that from patient No. 11 almost exclusively with dsDNA. However, in the majority of situations, reactivities with multiple antigens were seen. Ig from patient No. 6 reacted with ssDNA and RGG, No. 13 reacted with both ssDNA and dsDNA, Nos. 5 and 15 reacted with dsDNA and RGG, and, finally, Nos. 17 and 20 reacted with all three antigens tested. Thus, seven of these nine autoantibodies reacted as rheumatoid factors with varying degrees of DNA crossreactivities. The inability to precisely determine Ig contents in the various supernatants prevents us from making

TABLE III
Reactivities of Igs Produced by CD5⁺ B Cell Malignancies with Rabbit IgG, dsDNA, and ssDNA

Patient No.	Mitogen used	Reactivity with: Rabbit gamma globulin	ssDNA	dsDNA
4	T + PWM	0.42	0.00	0.00
5	T + PWM	1.21	0.50	2.34
6	PMA	5.12	6.59	0.26
7	T + PWM	2.85	0.00	0.09
8	T + PWM	2.28	0.00	0.00
9	PMA	0.00	0.00	0.00
10	T + PWM	0.67	0.00	0.00
11	PMA	0.33	1.27	16.61
12	T + PWM	0.42	0.00	0.00
13	PMA	0.08	6.72	3.70
14	PMA	0.00	0.00	0.00
15	T + PWM	1.41	0.21	2.29
16	PMA	0.00	0.00	0.00
17	T + PWM	50.38	4.87	15.04
18	T + PWM	0.85	0.00	0.00
19	T + PWM	0.00	0.00	0.00
20	PMA	1.56	1.23	3.21

Data for each antigen represent arbitrary binding units determined by dividing the OD units obtained for each sample by the OD units obtained using control human mAb reactive with RGG (Q39), ssDNA (W8), and dsDNA (W4), then multiplying by 10. Values >1.0 are considered significant. Irrelevant, monoclonal human Ig (up to 10 μg/ml) were assayed concomitantly and found to be negative.

accurate comparisons of relative binding avidity for the various autoantigens in the instances of multiple antigenic reactivity.

Monotypic Ig L Chains of Secreted Autoantibodies. To determine if the supernatant Ig that bound to the autoantigen(s) was indeed the monoclonal product of the CD5-expressing CLL or DWDL cells cultured, the L chain phenotypes of the autoantibodies were determined and compared with that found on the surface of the leukemic cells. The data in Table IV represent the results of companion EIA using the same supernatants as listed in Table III, developed with either goat anti-human κ or λ antibodies. Control human monoclonal Ig of different L chain types at concentrations up to 10 μg/ml were reacted with the L chain-specific goat anti-human antibodies and failed to demonstrate crossreactivity between these antibodies. As listed in Table III, there was a clear indication that the Ig binding to the autoantigen was monotypic as determined by L chain type. In all but one case, >99% of the autoantibodies detected by EIA displayed one L chain type. The only exception occurred with patient No. 20, the only case of DWDL studied. In this instance, λ antibodies predominated, although κ antibodies also were detectable. This result, however, correlates with the clinical situation since the patient's peripheral blood sample (white blood count, 10,200) was not a monoclonal B lymphocyte population. Although a lymph node that had been surgically removed for diagnostic purposes contained >65% $CD5^+$/sIgM λ^+ cells, the peripheral blood non-T cells displayed only a slight clonal excess of λ L chain-bearing non-T cells. The lack of peripheral B cell monoclonality is consistent with the patient's diagnosis since patients with DWDL usually have a monoclonal expansion of those B cells residing in the lymph nodes and less so in the circulating blood. In this and all the other cases, the L chain type detected on the surface of the overexpanded $CD5^+$ B cell clone agreed exactly with that detected on the autoantibody.

Similar cultures were established with non-T cells from 10 sex-matched and age-approximated normal individuals. The total Ig levels of these cultures ranged from 50 to 1,000 ng/ml (data not shown). As noted in Table V, the majority of normal individuals secreted Ig reactive with RGG, whereas only two showed insignificant

TABLE IV

Ig L Chain Expression of Autoantibodies Produced by $CD5^+$ B Cell Malignancies

Patient No.	sIg	RGG		ssDNA		dsDNA	
		κ	λ	κ	λ	κ	λ
5	γλ	<1.0	>99.0	<1.0	>99.0	ND	ND
6	μκ	>99.0	<1.0	>99.0	<1.0	ND	ND
7	μκ	>99.0	<1.0	ND	ND	ND	ND
8	μκ	>99.0	<1.0	ND	ND	ND	ND
11	μκ	ND	ND	>99.0	<1.0	>99.0	<1.0
13	γκ	ND	ND	>99.0	<1.0	>99.0	<1.0
15	μκ	>99.0	<1.0	ND	ND	ND	ND
17	μκ	>99.0	<1.0	>99.0	<1.0	>99.0	<1.0
20	μλ	33.0	67.0	14.0	86.0	31.0	69.0

Data are represented as the percent reactivity for a given Ig L chain type obtained by dividing the total OD units (at 490 nm) by the OD units obtained for the individual L chains. Enzyme immunoassays were performed with the same supernatants as depicted in Table III. However, one set was developed with affinity-purified goat anti-human κ and the other with goat anti-human λ antibodies to allow definition of L chain phenotypes of autoantibodies.

TABLE V

Reactivities with RGG, ssDNA, and dsDNA of Polyclonal Igs Secreted by PWM-stimulated B Lymphocytes from Normal Individuals

Normal donor	Age	Sex	Reactivity with: RGG	ssDNA	dsDNA
	yr				
1	39	M	1.95	0.00	0.00
2	61	F	0.45	0.00	0.00
3	53	F	0.60	0.00	0.00
4	59	M	2.82	0.00	0.00
5	69	M	1.94	0.00	0.00
6	53	F	2.36	0.00	0.00
7	65	M	4.39	0.65	0.00
8	57	M	4.86	0.00	0.53
9	58	M	0.01	0.00	0.00
10	58	M	1.21	0.00	0.00

Binding units determined as per Table III.

reactivity with ssDNA or with dsDNA. Unlike the situation with the patients with the lymphoproliferative disorders, all of these autoantibodies were polyclonal exhibiting both L chain types (data not shown).

Production of Autoantibodies by Pure Populations of CD5$^+$ B Cells. To confirm that the autoantibodies were the products of the CD5-expressing clone and not contaminating CD5$^-$ cells, non-T cells from patient No. 11 (which had been shown to secrete anti-DNA antibodies; Tables III and IV) were purified further by a FACS using an mAb reactive with CD5 and FITC-conjugated goat anti-mouse antibodies.

As shown in Fig. 1, before sorting, 98% of the non-T cells expressed CD5 (*A*); only a very small CD5$^-$ population existed, falling between channels 1 and 8. In addition, only 1.7% of the presorted cells expressed CD3 (Fig. 1 *B*), these cells falling

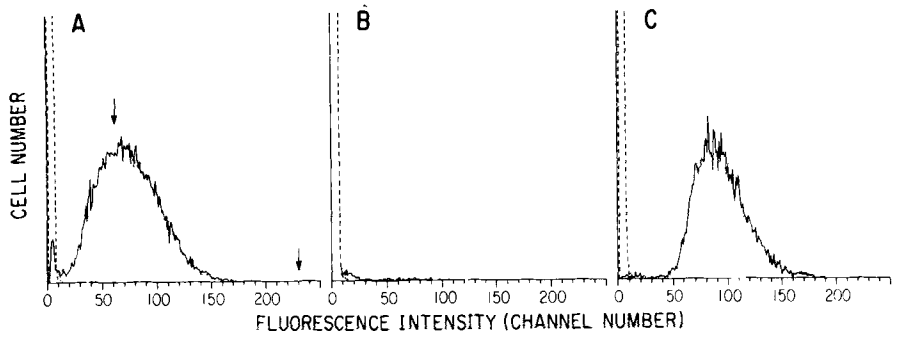

FIGURE 1. CD5 expression of leukemic non-T cells before and after cell sorting. Dotted lines represent results obtained with a control sample stained with an irrelevant mAb of the same isotype as the anti-CD5 mAb. Solid lines represent data obtained with the anti-CD5 mAb. (*A*) Histogram of indirect immunofluorescent staining of fresh, unsorted non-T cells from patient No. 11 with anti-CD5 mAb; percent CD5-reactive cells, 98%. Arrows illustrate the gates used for subsequent cell sorting. (*B*) Staining of unsorted non-T cells with anti-CD3 mAb; percent CD3-reactive cells, 1.7%. (*C*) Reanalysis of B lymphocytes for CD5 expression post sorting; percent CD5-reactive cells, 99.8%.

between channels 0 and 67. Sorting conditions were such that only those cells registering above channel 67 were collected, which represented the more intensely stained CD5-expressing B cells and excluded the CD5$^-$ cells and T cells. After sorting and before culturing, the isolated population was reanalyzed for CD5 expression. As shown in Fig. 1 *C*, 99.8% of the sorted cells expressed surface CD5 antigen.

These highly enriched CD5-expressing cells were cultured in the presence of PMA, the optimal differentiating agent in the initial set of experiments (Table II). Supernatants collected from this culture demonstrated that the sorted cells were induced to differentiate by PMA stimulation, although the levels of secreted Ig were less than those of the unsorted cells. Nevertheless, the supernatants from these cells contained anti-dsDNA antibodies (4.1 U as defined by the binding assay described in Table III) that displayed only κ L chains.

To rule out the possibility that the inability to detect autoantibody production in certain cultures was due to the presence of an insufficient number of CD5$^+$ cells, non-T cells from patient No. 12 were similarly sorted and stimulated in vitro to elaborate Ig. Neither the presorted nor sorted CD5-expressing B cell populations from this patient produced significant amounts of Ig reactive with either RGG, ssDNA, or dsDNA. Thus, it is clear that certain, but not all, clones of B cells expressing the CD5 antigen synthesize autoantibodies reactive with Ig or DNA.

Analyses of Serum for Autoantibodies from CLL/DWDL Patients. Serum from the CLL patients studied above and from normal controls were analyzed by EIA for reactivity with RGG, ssDNA, and dsDNA. In these sensitive enzyme immunoassays, all of the sera tested contained low and variable amounts of autoantibodies that were well below the levels seen in sera from patients with rheumatoid arthritis and SLE used as controls in the same assays (data not shown).

Discussion

The experiments presented in this study made use of two important features relating to the leukemic cells of CLL and related B cell lymphoproliferative disorders. First, the leukemic cells represent monoclonal expansions of CD5-expressing B lymphocytes (8–12) and second, they can be made to secrete antibody in vitro when stimulated with a battery of T cell-dependent and T cell-independent mitogens (reviewed in references 25 and 26). Thus, after 7 d in vitro, 17 of the 20 samples studied were found to elaborate significant amounts of Ig (Table II). Of these 17 Ig-producing B cell samples, 12 produced more Ig after stimulation with one or several of the T cell-independent mitogens PMA or SAC, and/or a T cell-dependent mitogen (PWM) in the presence of irradiated allogeneic T cells from normal individuals. These data are in agreement with prior studies demonstrating that CLL B lymphocytes can be made to differentiate to antibody-secreting cells when appropriately stimulated in vitro. This was initially demonstrated by Fu et al. (27) in selected CLL patients with serum monoclonal gammopathies. With the aid of heterologous anti-idiotypic antibodies, these investigators showed that the CLL leukemic cells could be differentiated to plasma cells by providing normal T helper function. Subsequently, Robert et al. (28) showed that by using a battery of T cell-dependent and T cell-independent mitogens, B cells from the majority of CLL patients could be induced to secrete antibodies with monotypic L chains identical to those displayed on the leukemic cell surface.

The antibodies produced by the Ig-secreting cells in this study were assayed by EIA for reactivity with ssDNA, dsDNA, and IgG. A surprisingly high number of patients (>50%; 9:17) were found to synthesize Ig reactive with one or several of these autoantigens. As shown in Table III, antibodies from five patients were reactive with ssDNA, six with dsDNA, and seven with RGG (RFs). RGG was used as the target in the RF immunoassays since it allowed the determination of IgG reactivity without requiring isolation and labeling of the RF from the culture supernatants. It should be mentioned that although certain human RFs react only with human IgG or with only rabbit IgG, most react with both (20–23). The possibility that in certain instances autoantibodies were not detected by our assays because the leukemic cells did not produce sufficient amounts of Ig also cannot be ruled out. Indeed, cultures from patients Nos. 4, 10, 12, and 18 demonstrated low levels of RF activity that were not considered significant in our arbitrary binding unit system. Experiments are currently underway to create hybridomas with $CD5^+$ cells from selected patients that will allow collection of larger quantities of Ig to test these hypotheses. Furthermore, additional autoreactive clones might be detected among these patients if a more comprehensive autoantigen panel is used. This is also presently being tested.

The autoantibodies secreted by the patients' B cells displayed monotypic Ig L chains that were identical to that expressed on the surface of the $CD5^+$ leukemic cells (Table IV), strongly suggesting that they were the products of the CD5-expressing clone. Nevertheless, to rule out the unlikely possibility that contamination with $CD5^-$ B cells was responsible for the autoantibody production observed, the non-T cell fraction from patient No. 11 was purified further by a FACS into >99.8% $CD5^+$ B cells. Supernatants from mitogen-stimulated cultures of these cells reacted strongly with dsDNA, demonstrating the production of autoantibodies by this $CD5^+$ B cell clone. Thus, the autoantibodies detected in these cultures were the clonal products of the CD5-expressing B cells.

As expected, similar cultures of non-T cells from normal volunteers produced higher levels of Ig compared with the CLL cultures. Most of these supernatants exhibited RF activity with minimal ssDNA or dsDNA reactivity (Table V), a finding in agreement with studies described by other investigators (29, 30). These RFs were polyclonal antibodies since they exhibited both κ and λ L chain usage. However, despite the relatively high levels of Ig in the supernatants, the RF activity in several instances was lower than that seen for the CLL samples. This most likely reflects the fact that only a minority of the normal peripheral blood non-T cells are $CD5^+$ whereas the majority are $CD5^-$ (31, 32). Therefore, most of the Ig secreted by the normal cells probably is derived from the $CD5^-$ population and is reactive with conventional antigens, while a smaller amount comes from the $CD5^+$ cells and reacts with autoantigens. A more relevant set of control cells for our study would be monoclonal populations of $CD5^-$ B cells. We have been able to study only a limited number of patients with $CD5^-$ B cell malignancies to date (four patients with hairy cell leukemia). Although the number of patients evaluated is too small to draw firm conclusions, so far, Ig from cultures of these cells have not shown autoreactivity.

The data in the present study are consistent with experiments performed in mice demonstrating that Ly-1^+ B lymphocytes preferentially produce antibodies reactive with autologous erythrocytes, thymocytes, IgG, or ssDNA (3, 6, 7). Studies in man have shown that higher than normal numbers (31–37) of CD5-expressing cells can

be found in selected patients with autoimmune disorders (38, 39). In addition, our data support and extend the observations of Casali et al. (31) and Hardy et al. (32) showing that enriched polyclonal populations of CD5$^+$ B cells, taken from normal peripheral or umbilical cord blood, secrete autoantibodies after EBV infection or SAC stimulation. However, several features and findings make the studies reported here distinct from those just mentioned.

First, only in the present study have monoclonal populations of human CD5$^+$ B cells been used to demonstrate autoantibody production, definitively illustrating that the autoantibodies are products of the CD5-expressing B cells. Second, the specificities of the autoantibodies detected deserve special comment. Six patients' B cells secreted mAbs reactive with dsDNA (Table III), a set of autoantibodies that usually correlates best with autoimmune symptomatology and that has been considered relatively specific for SLE (40). To date, dsDNA production by polyclonal populations of CD5$^+$ or Ly-1$^+$ B cells has not been reported.

Certain of these dsDNA-reactive autoantibodies also behaved as RFs (patients Nos. 5, 15, 17, and 20). One of these dsDNA/RF mAbs appears to bind IgG better than dsDNA (No. 17). This autoantibody may be a monoclonal representation of the subset of mAb shown to bind IgG and DNA-histone complexes, an RF group that displays a unique crossreactive idiotype (41). Since the other three dsDNA/RF mAbs (Nos. 5, 15, and 20) appear to bind dsDNA more effectively, they probably do not fall into this group. In addition, it is noteworthy that certain of the RFs secreted by our patients contained λ L chains (Nos. 5 and 20), an L chain type infrequently associated with RFs clinically but overutilized by Ly-1$^+$ B cells in the mouse (7). Our demonstration that certain patients with CLL secrete RF provides confirmatory evidence for the studies of Preud'Homme and Seligmann (42), showing that the expanded B cell clone of selected patients with lymphoproliferative disorders can bind IgG to surface membrane-bound IgM molecules.

Finally, previous studies in mouse (3, 5–7) and man (32, 33, 35, 36) have suggested that most, if not all, of the autoreactive Ly-1/CD5$^+$ clones display an IgM$^+$/IgD$^+$ surface phenotype. In addition, the autoreactive antibodies generated by CD5$^+$ human B cells are usually of the IgM isotype (32, 43). In the present study, CD5$^+$ cells from four patients (Nos. 5, 10, 13, 19) synthesized IgG and two of these four produced autoantibodies reacting with either dsDNA and IgG (No. 5) or ssDNA and dsDNA (No. 13). Thus, these patients illustrate that CD5$^+$/IgG$^+$ cells exist in man and certain clones with this phenotype can produce IgG autoantibodies.

Although the autoantibodies produced by the CD5$^+$ B cells in this study are monoclonal, the majority of these mAb are "polyspecific," binding to a number of different autoantigens (Table III). Such polyspecific autoantibodies have been described previously in human (44) and murine (45) sera, as well as among human monoclonal autoantibodies (43, 46).

The preceding data may help to understand certain clinical observations about patients with CLL. Since our data demonstrate that B cells from a remarkably high percentage (>50%) of patients with CD5-expressing lymphoproliferative disorders synthesize autoreactive antibodies, this may relate to the findings that the sera of some patients display autoantibodies or that certain patients develop frank autoimmune problems, such as autoimmune hemolytic anemia (13). However, considering

that all patients with CLL develop an overexpansion of the CD5-expressing B cell lineage, it is surprising that many more patients do not experience autoimmune complications or have high levels of autoantibodies in their sera. The absence of these phenomena may be a reflection of either a lack of correlation between these phenomena and the expanded $CD5^+$ clone and/or a specific differentiation block whereby only a few cells of the malignant clone undergo terminal maturation to antibody-secreting cells. This failure to terminally differentiate may be due, in part, to a Th defect (47).

Finally, the presented data are consistent with the notion that the autoreactivity of $CD5^+$ B cells may play a role in the clonal expansion seen in these lymphoproliferative disorders. Repetitive crosslinking of surface Ig receptors by autoantigens may lead to proliferation and expansion of the $CD5^+$ clone. Because of the differentiation block mentioned above, this clone may not mature to plasma cells that could induce negative feedback signals. The high incidence of B cell malignancies in mice (48) and in humans (49, 50) with autoimmune diseases may in part be a reflection of this or a related mechanism.

Summary

CD5-expressing B lymphocytes from patients with selected chronic lymphoproliferative disorders were used to determine whether monoclonal populations of $CD5^+$ human B cells produce autoantibodies. $CD5^+$ B cells from 19 patients with chronic lymphocytic leukemia (CLL) and one with diffuse well-differentiated lymphocytic lymphoma (DWDL) were cultured, with and without mitogenic stimulation, to obtain Ig from these cells. 17 of the 20 samples produced Ig in vitro. mAb from nine of the 17 patients were reactive with either IgG, ssDNA, or dsDNA. In every instance, the autoantibodies displayed monotypic L chain usage that correlated precisely with the L chain expressed on the $CD5^+$ leukemic B cell surface. These monoclonal autoantibodies varied in their degree of antigenic specificity; some were quite specific, reacting with only one antigen, whereas others were polyspecific, reacting with two or all three autoantigens tested.

Three features distinguish these autoantibodies from those observed in prior studies of $CD5^+$ B cells. First, they are clearly the products of monoclonal populations of $CD5^+$ cells; second, several react with dsDNA, a specificity not previously reported and often seen in association with significant autoimmune disorders; and third, two of the monoclonal autoantibodies secreted by the $CD5^+$ clones were of the IgG class. Although not all of the Ig-producing, CD5-expressing clones elaborated mAbs reactive with the autoantigens tested, >50% did. It is possible that with a broader autoantigenic panel or with larger quantities of CLL/DWDL-derived Ig, even more autoantibody-producing clones might be identified. These studies may have important implications for the antigenic specificity of subsets of human B lymphocytes as well as for lymphoproliferative and autoimmune disorders in general.

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