Defective T Cell-Mediated, Isotype-Specific Immunoglobulin Regulation in B Cell Chronic Lymphocytic Leukemia

By Jonni S. Moore, Michael B. Prystowsky, Richard G. Hoover, Emmanuel C. Besa, and Peter C. Nowell

The consistent occurrence of T cell abnormalities in patients with B cell chronic lymphocytic leukemia (B-CLL) suggests that the non-neoplastic host T cells may be involved in the pathogenesis of this B cell neoplasm. Because potential defects of immunoglobulin regulation are evident in B-CLL patients, we investigated one aspect of this by studying the T cell-mediated immunoglobulin isotype-specific immunoregulatory circuit in B-CLL. The existence of class-specific immunoglobulin regulatory mechanisms mediated by Fc receptor-bearing T cells (FcR+ T) through soluble immunoglobulin binding factors (IgBFs) has been well established in many experimental systems. IgBFs can both suppress and enhance B cell activity in an isotype-specific manner. We investigated the apparently abnormal IgA regulation in a B-CLL patient (CLL249) whose B cells secrete primarily IgA in vitro. Enumeration of FcR+ T cells showed a disproportionate increase in IgA FcR+ T cells in the peripheral blood of this patient. Our studies showed that the neoplastic B cells were not intrinsically unresponsive to the suppressing component of IgABF produced from normal T cells, but rather the IgABF produced by the CLL249 host T cells was defective. CLL249 IgABF was unable to suppress IgA secretion by host or normal B cells and enhanced the in vitro proliferation of the host B cells. Size fractionation of both normal and CLL249 IgABF by gel-filtration high-performance liquid chromatography (HPLC) demonstrated differences in the ultraviolet-absorbing components of IgABF obtained from normal T cells that from our patient with defective IgA regulation. Such T cell dysfunction may not be restricted to IgA regulation, since we have found similar expansion of isotype-specific FcR+ T cells associated with expansion of the corresponding B cell clone in other patients with B-CLL. These data suggest that this T cell-mediated regulatory circuit could be significantly involved in the pathogenesis of B-CLL.
peripheral blood is often difficult, normal B cells were freshly isolated from tonsils immediately after surgical removal. Tonsils were obtained from both male and female donors ranging in age from 4 to 13 years and were infection-free at time of surgery. Normal T cells were isolated from the peripheral blood of healthy, age-matched donors. These investigations were performed after approval by the Committee on Studies Involving Human Beings of the University of Pennsylvania.

Cell lines. GM1056A, a chromosomally normal lymphoblastoid cell line that secretes IgA2, and GM1500B, an Epstein-Barr virus (EBV)-transformed lymphoblastoid cell line that secretes IgG2K, were obtained from the Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camden, NJ. These cell lines were maintained in our laboratory by serial passage in RPMI 1640 supplemented with 15% fetal calf serum (FCS).

Isolation of T and B cells. Blood from B-CLL patients or normal individuals was enriched for lymphocytes by Ficoll-Hypaque separation.28 T cells were purified from this population by two sequential rosetting procedures with neuraminidase-treated sheep erythrocytes followed by Ficoll-Hypaque separation according to the procedure of Moretta et al.34 The T-enriched (rosetted) fractions contained ≥95% T cells as determined by staining with OKT11 (Ortho Diagnostic Systems, Raritan, NJ). For both CLL and normal preparations, the nonrosetted (fraction contained ≥98% B cells, as determined by the presence of surface immunoglobulin and/or by positive staining by OK*1 antibody (anti-human DR framework). Although monocytes were not specifically removed from these preparations, contamination by this population was minimal. In most B-CLL B cell populations, the level of Leu 1-positive cells was equal to or higher than that of OK1α1-positive cells, indicating that the OK1α1-positive population was essentially monocyte-free. In normal B cell populations, staining with OKB7 indicated monocyte contamination of <1.5%.

Immunofluorescent determination of lymphocyte phenotype. Phenotypes of lymphocyte populations were assessed by immunofluorescence and flow cytometry. In brief, 10⁶ cells were incubated with an appropriate dilution of antibodies OKT3, OKT11, OKT4, OKT8, OKB7, OK α1*1 (Ortho Diagnostic Systems); anti-Leu 1, anti-human IgA, IgG, IgM, or IgD and anti-human x or A light chain (Becton Dickinson, Mountain View, CA) in 200 μL Dulbecco's phosphate-buffered saline (DPBS), pH 7.4, with 2% bovine serum albumin (BSA) and 0.02 NaCl (staining buffer) at 4°C for 30 minutes. After three washes, the cells were resuspended in 100 μL staining buffer and 100 μL FITC-conjugated goat anti-mouse IgG diluted 1:20 (Tago, Burlingame, CA). This suspension was further incubated at 4°C for 30 minutes, followed by three washes with staining buffer. The cells were resuspended in staining buffer and analyzed on an Ortho Spectrum III cytofluorograph.

Measurement of FeR-positive T cells. Feα receptors were measured on T cells by using a hapten-specific rosette assay: 5 × 10⁶ T cells in RPMI with 20% FCS were incubated with 500 μg IgA anti-TNP antibody for 1 hour at 37°C to occupy Feα receptors.40 This was followed by rosetting with TNP-coated or erythrocytes: TNP was coupled to ox erythrocytes as previously described.41 Rosettes were formed by sedimenting the T cells in 100 μL with 100 μL 1% haptenated ox erythrocytes (ORBCs) at 4°C for 1 hour. Feα receptor-bearing T cells were enumerated by counting the number of lymphocytes with three or more bound erythrocytes.

T cells with Fe receptors for IgG and IgM were detected as described.42 In brief, ORBCs were sensitized with optimal dilutions of purified IgG or IgM rabbit anti-ORBC (Cooper Biomedical, Malvern, PA) at 37°C for 45 minutes. For detection, 100 μL T cell suspension (2 × 10⁶ total cells) was mixed with 100 μL 1% IgG-ORBC, pelleted, and incubated on ice for 30 minutes. Fe-μ receptor-positive T cells were enumerated by counting the number of rosettes formed with IgM-ORBCs, and Fe-γ receptor-positive T cells were determined by rosette formation with IgG-ORBCs.

In vitro cell culture. To determine the effects of IgABF on immunoglobulin synthesis and secretion in vitro, 1 × 10⁶ B cells plus 1 × 10⁶ T cells from normal subjects or from B-CLL patients were cultured with 0.75 μg pokeweed mitogen (PWM) in RPMI 1640 supplemented with 10% heat-inactivated FCS, 10 mmol/L L-glutamine, 1% penicillin-streptomycin (RPMI complete medium, CM) for 8 days with or without IgBFs. These culture conditions had been previously determined by titration to be optimal for PWM-induced immunoglobulin synthesis and secretion in our laboratory. The cell-free supernatants of each culture were collected individually and assayed for IgM, IgG, and IgA production by enzyme-linked immunosorbent assay (ELISA).

ELISA for immunoglobulin production. A modified ELISA technique was used to determine the Ig content of the culture-generated supernatants. One-half area flat-bottom 96-well microtiter plates (Costar, Cambridge, MA) were precoated at 4°C overnight with either goat anti-human IgM, IgG, or IgA of previously determined dilution in 0.1 mol/L sodium carbonate buffer. The plates were then blocked with 1% BSA in PBS for 1 hour at room temperature, followed by thorough washing with PBS plus 0.05% Tween 20. Each plate was then set up with the following controls: (a) blank-PBS/Tween; (b) RPMI CM alone; (c) dilutions of standard human IgM, IgA, or IgG preparations (Tago) ranging from 1 × 10⁻⁴ to 2.5 × 10⁻¹ μg/μL prepared in RPMI CM. Unknown samples were run in triplicate undiluted and at 1:10 and 1:100 dilutions. The plates were then incubated at room temperature for 3 hours and subsequently washed thoroughly with PBS-Tween. Horseradish peroxidase conjugated goat anti-human IgG, IgM, or IgA (dilution determined by titration) was added to all wells except blanks and incubated at 4°C overnight. The following day, the plates were washed and the enzyme substrate (0.4% O-phenylenediamine plus 0.012% H₂O₂ in phosphate citrate buffer, pH 5) was added to all wells. Plates were read at 492 nm when color developed. The means of triplicate readings were calculated, and the experimental values were determined by comparison to the standard IgM, IgG, and IgA curve values using computer-assisted linear-regression analysis. In our laboratory, Ig secretion from normal tonsil B cells stimulated with PWM is highly variable. The following ranges can be considered within the limits seen with B cells from control donors: IgM, 107 to 17,100 ng/mL; IgG, 298 to 40,000 ng/mL; and IgA, 43 to 3,180 ng/mL.

Production of IgABF. Human IgABF was produced using a modification of a recently described protocol.35 Normal or B-CLL T cells were obtained as described above and cultured at 6 × 10⁶ in 2 mL RPMI CM at 37°C for 2 days. Four hundred micrograms purified human secretory IgA (Cooper Biomedical, Malvern, PA) was added to each culture, and the incubation was continued for another 24 hours. The T cells were then harvested, washed free of IgA, and recultured for an additional 24 hours in RPMI CM alone. The culture conditions for inducing human IgABF were based on similar studies with murine T cells and on a limited titration of normal human T cells with secretory IgA. The cell-free supernatants were collected, concentrated by lyophilization, and resuspended to 10% of the original volume in RPMI. Then 0.5 mL of this product was incubated on a human IgA Sepharose CL-4B affinity column (5 mg IgA/1 mL swollen beads/column) for 3 hours at room temperature. The effluent was washed through with 20 mL PBS. The columns were then eluted with 10 mL 0.1 mol/L glycine-HCl, pH 3, for 1 hour at room temperature. The acid eluates were lyophilized, resuspended to 1 mL, and dialyzed against RPMI 1640. These eluates were designated IgABF.

DNA synthesis in human B cells. The effects of IgABF on cell proliferation were assessed by measuring [3H]-thymidine incorpora-
tion by the B cells. Freshly isolated normal or B-CLL B cells (2 x 10^5 in 0.2 mL) were cultured with 10 μL of a 1:1000 dilution of Staphylococcus aureus (Pansorbin, Calbiochem, San Diego) and IgABF from either normal or B-CLL T cells. Cultures were incubated in 96-well flat-bottom microtiter plates (Falcon) in 5% CO₂ and air at 37°C for 2 to 4 days. Lymphoblastoid cell lines GM1056A and GM1500B were similarly cultured, without mitogen, with or without IgABF. Eight hours prior to termination of incubation, cells were harvested in 0.2 mL) were cultured with 10μM of PWM in 22 of 27 experiments using cells from 25 different patients (ranges 6.7 to 618 ng/mL IgM, 5 to 1,250 ng/mL IgG, and 5.6 to 559 ng/mL IgA). In experiments with six different patients, all hypogammaglobulinemic, we demonstrated that when detectable amounts of IgA were produced by PWM-stimulated B-CLL B cells, the response could be specifically suppressed by IgABF from normal T cells. The mean suppression of the IgA response was 58 (range 27% to 100%), significant when compared with that of the IgM or IgG responses (P < 0.03) and indicating that B-CLL B cells are responsive to normal isotype regulatory factors. The responding B cells in these experiments were indeed the neoplastic B cells, since >97% of the circulating B cells in the B-CLL patients were Leu 1-positive. The Leu 1 marker is rare in normal B cells but is commonly highly expressed on B-CLL B cells.43

Some suppression of the IgG response was also occasionally seen, but the major effect was on the IgA secretion of B cells from either normal donors or B-CLL patients. Revillard et al.44 recently reported that a nonisotype-specific suppressor factor contaminates some preparations of IgA binding factor; this may explain the inconstant suppression of IgG observed in some of the experiments in this report.

Elevation of IgA Fc receptor-bearing T cells in a patient with B-CLL. We next investigated a patient (CLL249) with long-term, indolent, untreated B-CLL whose lymphocytes secrete primarily IgA in vitro, with little or no IgM or IgG. This patient's serum had no detectable immunoglobulin. Flow cytometry indicated that 98% of the CLL249 lymphocytes were La-positive, 94% were Leu 1-positive, and <4% expressed detectable levels of surface IgM,G,A,D, or κ or λ light chains. Chromosomal analysis indicated a clonal population having a pseudodiploid karyotype with a t(14;22) translocation.37 This patient was quite unusual since most CLL B cells secrete IgM or IgG with PWM stimulation in vitro.

We first determined the frequency of FcR + T cells in the peripheral blood of CLL249 (Table 1). Our analysis indicated a significant increase in the percentage of T-α cells (17.5%), as compared with normal mononuclear cells (PBMCs) (3.8%) or tonsillar (5.2%) lymphocyte populations, with normal levels of T-α and T-γ cells. Although some investigators have reported higher levels of T-γ cells in normal individuals,45 the levels of ≤5% indicated in Table 1 are consistent with previous findings by one of us (R.H.) and by other researchers.46 We investigated the IgA isotype

**Fig 1.** Suppression of IgA secretion from normal human B cells by normal IgABF. Tonsil B cells 1 x 10^6 were cultured with 1 x 10^6 autologous T cells with or without 0.4% IgABF and/or 0.76 μg PWM for 8 days. Cell-free supernatants were collected and analyzed for Ig content by ELISA. Percentage suppression = 1 - (B + T + PWM + IgABF/B + T + PWM) ÷ B + T + PWM. Data represent the median and range of suppression of eight separate experiments. Significance was determined by Mann-Whitney test.
IgA REGULATION IN B-CLL

Table 1. Elevation of IgA FcR + T Cells in the Peripheral Blood of a B-CLL Patient

<table>
<thead>
<tr>
<th>Cell Source†</th>
<th>Secreted Ig‡</th>
<th>FcR + T Cells (%)†*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL249 IgA</td>
<td>22.5 ± 0.7</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Normal PBMC</td>
<td>32.6 ± 1.8</td>
<td>0.8 ± 0.6</td>
</tr>
<tr>
<td>Normal tonsil</td>
<td>41.4 ± 4.3</td>
<td>5.3 ± 4.4</td>
</tr>
</tbody>
</table>

†Peripheral blood lymphocytes from a normal donor (WBC 5,000) or B-CLL patient 249 (WBC 18,000) or normal tonsil lymphocytes were separated into B and T cell fractions by Ficoll-Hypaque.

Table 2. Normal IgABF Suppresses IgA Secretion by CLL249 B Cells

<table>
<thead>
<tr>
<th>Culture Condition</th>
<th>IgM (ng/mL)</th>
<th>IgG (ng/mL)</th>
<th>IgA (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL249 + T</td>
<td>15.1</td>
<td>78.2</td>
<td></td>
</tr>
<tr>
<td>CLL249 + T + PWM</td>
<td>11.9 (21)</td>
<td>17.1 (78)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Failure of CLL249 IgABF to Suppress IgA Secretion by Normal B Cells

<table>
<thead>
<tr>
<th>Culture Condition</th>
<th>IgM (ng/mL)</th>
<th>IgG (ng/mL)</th>
<th>IgA (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B + T</td>
<td>38.3</td>
<td>885.0</td>
<td>93.0</td>
</tr>
<tr>
<td>B + T + PWM</td>
<td>107.0</td>
<td>1061.0</td>
<td>231.0</td>
</tr>
<tr>
<td>B + T + PWM + CLL249 IgABF</td>
<td>148.0 (0)</td>
<td>891.0 (16)</td>
<td>224.0 (3)</td>
</tr>
<tr>
<td>B + T + PWM + normal IgABF</td>
<td>107.0 (0)</td>
<td>910.0 (14)</td>
<td>137.7 (41)</td>
</tr>
</tbody>
</table>

regulatory circuit in CLL249 by examining the IgABF produced by the patient's own T cells and the response of her neoplastic B cells to normal IgABF.

Normal IgA binding factor suppresses IgA secretion by CLL249 B cells. Freshly isolated B cells from patient CLL249 were cocultured with autologous T cells and PWM with or without 0.4% IgABF produced from the T cells of a normal donor (Table 2). This concentration of IgABF had been shown previously (Fig 1) to be effective in suppressing IgA secretion by B cells from normal donors.

Indeed, in four of five experiments performed with B cells from different normal donors, enhancement of IgA secretion was noted with CLL249 IgABF.

We next tested the CLL249 IgABF on the B cells from another B-CLL patient (Table 4). The addition of CLL249 IgABF to these CLL B cells resulted in a marked potentiation of IgA secretion by cells normally producing extremely low levels of IgA in three of five experiments, each with cells from different patients.

CLL249 IgABF enhanced IgA secretion by autologous B cells. We also tested the effect of CLL249 IgABF on the in vitro IgA secretion by the autologous CLL249 B cells (Table 5). Although normal IgABF caused only slight suppression in this particular experiment, CLL249 IgABF had no suppressive effect on autologous IgA secretion and actually enhanced the response in this and two other experiments using CLL249 B cells isolated from the patient at different times. Thus, in all three systems tested (normal, neoplastic, autologous), CLL249 IgABF was unable to suppress IgA secretion and indeed often enhanced this response.

CLL249 IgABF stimulated the proliferation of IgA-committed B cells. Although the effects of IgABF on immunoglobulin secretion are important, a more critical question for the leukemic patient is the effect of such factors on the proliferation of the neoplastic clone. B cells from CLL249, purified by two rounds of E rosetting, were...
cultured with the T-independent B-cell mitogen *S. aureus* and with either 0.4% CLL249 IgABF or normal IgABF. For these initial studies, IgABF was tested at the same concentration that had inhibited IgA synthesis in CLL249 B cells. Proliferation was determined by $^3$H-thymidine incorporation on days 2, 3, and 4 of culture (Fig 2). The *S. aureus*-stimulated proliferation of CLL249 B cells was significantly suppressed by two different preparations of normal IgABF on days 3 and 4 of culture (both $P < .05$). In contrast, CLL249 IgABF was markedly stimulatory, approximately doubling the uptake of $^3$H on day 2, and indicating a significantly greater number of responding cells. A limited titration was performed with CLL249 IgABF, demonstrating significant enhancement of proliferation by concentrations ranging from 0.4% to 10% with no detectable suppression. No differences in cell viabilities or in the high frequency of Leu 1-positive cells were detected in the different experimental conditions.

To determine if the effects of CLL249 IgABF on B cell proliferation were isotype-specific, we cultured normal IgABF or CLL249 IgABF with GM1056A (an IgA-producing lymphoblastoid line) or GM1500Ban (an IgG-producing lymphoblastoid line) cells. Proliferation was assessed as above by $^3$H-thymidine incorporation on days 1, 2, and 3 (Table 6). Whereas normal IgABF consistently suppressed the proliferation of the IgA-producing cells (GM1056A) by as much as 56% in the first 24 hours of culture, no suppression was evident with CLL249 IgABF, and indeed there was significant stimulation of proliferation of these cells by day 2 of culture. Neither factor was able to effect significant suppression (or enhancement) of the IgG-producing cells (GM1500B) until day 3, when there was slight inhibition with both factors. Thus, the effects of both CLL249 and normal IgABF on B cell proliferation appeared to be predominantly IgA isotype-specific, differing only with respect to their regulation of IgA-committed B cells.

**CLL249 and normal IgABF exhibit different HPLC elution profiles.** As an initial step in the biochemical characterization of normal IgABF, IgA affinity column eluates were applied to a gel filtration column, previously standardized with proteins of known mol wt. The elution profile obtained with IgABF derived from normal, IgA-stimulated T cells (Fig 3A) was compared with that obtained using

| Table 4. Failure of CLL249 IgABF to Suppress IgA Secretion by B-CLL B Cells |
|---------------------------------|-----------------|-----------------|-----------------|
| Cultures*                                 | IgM (ng/mL) | IgG (ng/mL) | IgA (ng/mL) |
| B-CLL B + B-CLL T                       | 8.7          | 8.8           | 0             |
| B-CLL B + B-CLL T + PWM                 | 234.0        | 99.3          | 60.6          |
| B-CLL B + B-CLL T + normal IgABF        | 215.0 (8)    | 74.9 (25)     | 0 (100)       |
| B-CLL B + B-CLL T + PWM + 249 IgABF     | 276.0 (0)    | 117.9 (0)     | 149.0 (0)     |

*B cells 1 x 10⁶ and autologous T cells 1 x 10⁶ from a separate B-CLL patient (WBC 15,600) were cultured with or without 0.75 μg PWM and 0.4% normal or CLL249 IgABF.

†Cell-free supernatants were analyzed for Ig content by ELISA 8 days later. Results are representative of five separate experiments.

**CLL249 and normal IgABF exhibit different HPLC elution profiles.** As an initial step in the biochemical characterization of normal IgABF, IgA affinity column eluates were applied to a gel filtration column, previously standardized with proteins of known mol wt. The elution profile obtained with IgABF derived from normal, IgA-stimulated T cells (Fig 3A) was compared with that obtained using

| Table 5. Failure of CLL249 IgABF to Suppress IgA Secretion by Autologous B Cells |
|---------------------------------|-----------------|-----------------|
| Cultures*                                 | IgM (ng/mL) | IgG (ng/mL) | IgA (ng/mL) |
| CLL249 B + CLL249 T               | 0             | 0             | 21.6         |
| CLL249 B + CLL249 T + PWM         | 6.7           | 13.1          | 81.9         |
| CLL249 B + PWM + CLL249 IgABF     | 7.1 (0)       | 17.3 (0)      | 113.0 (0)    |
| CLL249 B + CLL249 T + PWM + normal IgABF | 12.3 (0) | 15.2 (0)     | 68.1 (17)    |

*B cells 1 x 10⁶ and autologous T cells 1 x 10⁶ from patient CLL249 were cultured alone, with 0.75 μg PWM, and/or 0.4% normal or CLL249 IgABF.

†Cell-free supernatants were analyzed for Ig content after 8 days of culture.
Fig 3. Gel filtration HPLC of (A) normal and (B) CLL249 IgABF. Concentrated IgABF was applied to a sizing column equilibrated in DPBS (pH 7.0) that had been standardized with proteins of known mol wt. Fractions were collected at 0.5-mm intervals and monitored at 280 nm (scales on ordinate are different).

Table 6. Effects of IgABF on Proliferation Are Isotype-Specific

<table>
<thead>
<tr>
<th>Cultures*</th>
<th>cpm</th>
<th>% Suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM1056A alone</td>
<td>16,996</td>
<td>25,335</td>
</tr>
<tr>
<td>GM1056A + 0.4% CLL249 IgABF</td>
<td>18,340 (0)</td>
<td>34,602 (0)</td>
</tr>
<tr>
<td>GM1056A + 0.4% normal IgABF</td>
<td>7,400 (56)</td>
<td>23,204 (6)</td>
</tr>
<tr>
<td>GM1500B alone</td>
<td>12,331</td>
<td>13,373</td>
</tr>
<tr>
<td>GM1500B + 0.4% CLL249 IgABF</td>
<td>10,518 (14)</td>
<td>13,866 (0)</td>
</tr>
<tr>
<td>GM1500B + 0.4% normal IgABF</td>
<td>13,232 (0)</td>
<td>11,639 (22)</td>
</tr>
</tbody>
</table>

*GM1056A or GM1500B cells 1 x 10⁶ were cultured alone or with 0.5% normal or CLL249 IgABF for 1, 2, or 3 days. Eight hours prior to harvest, 2 µCi ³H-thymidine was added to each well.

†Proliferation was assessed by ³H-thymidine incorporation in cpm. Values represent means of triplicate cultures.

IgABF produced in the same manner by the CLL249 T cells (Fig 3B). Several differences were noted in the two profiles. Normal IgABF contained four to five protein peaks ranging in mol wt from 8,000 to 10,000 to 70,000. The two higher mol-wt peaks were markedly reduced in the CLL249 IgABF, whereas the smaller sized components were similar in both preparations. The total protein detected in normal IgABF was much higher than that detected in CLL249 IgABF produced from the same number of T cells. Therefore, it appeared that both quantitative and qualitative differences between normal and CLL249 IgABF could be detected by gel filtration. The biologic activities of each individual protein peak are currently being investigated.

Increase in FcR+ T cells in B-CLL patients corresponds with the isotype of the major secreted immunoglobulin in vitro. To begin to determine if defective isotype regulation is a universal characteristic of B-CLL, we analyzed the levels of FcR+ T cells in nine B-CLL patients and correlated these numbers with the isotype of the major immunoglobulin secreted in vitro (Table 7). We defined the major immunoglobulin secreted in vitro as the isotype present in highest absolute concentration in supernatants of CLL B cells stimulated by PWM for 8 days. In seven of nine cases, abnormally high levels of a particular FcR+ T cell subset were observed, and these correlated with the isotype of the major immunoglobulin secreted in vitro. Those patients secreting increased

Table 7. Increase in FcR+ T Cells in B-CLL Corresponds With Isotype of Primary Secreted Immunoglobulin

<table>
<thead>
<tr>
<th>Patient</th>
<th>Lymphocyte Markers</th>
<th>Primary Secreted lg*</th>
<th>FcR+ T Cells (%)†</th>
<th>WBC§</th>
<th>RAI¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>249</td>
<td>No sig. 10% T</td>
<td>IgA</td>
<td>22.5</td>
<td>0</td>
<td>20.5</td>
</tr>
<tr>
<td>440</td>
<td>M, &lt;10% T</td>
<td>IgG</td>
<td>13.3</td>
<td>21.0</td>
<td>5.5</td>
</tr>
<tr>
<td>306</td>
<td>M, 3% T</td>
<td>IgM + IgG</td>
<td>14.2</td>
<td>22.5</td>
<td>5.9</td>
</tr>
<tr>
<td>309</td>
<td>18% T</td>
<td>0</td>
<td>13.0</td>
<td>4.2</td>
<td>3.0</td>
</tr>
<tr>
<td>242</td>
<td>G, 33% T</td>
<td>IgG</td>
<td>13.0</td>
<td>27.0</td>
<td>2.0</td>
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<tr>
<td>247</td>
<td>0</td>
<td>IgG + IgA</td>
<td>13.5</td>
<td>9.3</td>
<td>9.6</td>
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<tr>
<td>467</td>
<td>M, 11% T</td>
<td>IgM + IgG</td>
<td>15.0</td>
<td>7.6</td>
<td>1.5</td>
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<tr>
<td>350</td>
<td>Low sig. 11% T</td>
<td>IgM, IgG, IgA</td>
<td>15.5</td>
<td>4.6</td>
<td>5.6</td>
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<tr>
<td>498</td>
<td>40% T</td>
<td>IgG</td>
<td>0</td>
<td>18.5</td>
<td>4.3</td>
</tr>
</tbody>
</table>

*Isotype and amount of in vitro PWM-stimulated Ig secretion determined by ELISA.
†FcR+ T cells determined by rosette formation with IgM- or IgG-coated ox erythrocytes. FcR-α T cells were measured by incubating T cells with anti-TNP specific IgA and then rosetting with TNP-coated ox erythrocytes. Normal ranges in our laboratory are T-µ 20% to 35%, T-γ 0% to 5%, T-α 3% to 5%. Abnormal values are underlined.
‡No sig was demonstrated by flow cytometry; however, after biosynthetic labeling with ³⁵S-methionine, membrane-associated α heavy chains and κ light chains were detected by SDS-PAGE analysis.
§The patient’s WBC count and RAI clinical classification at the time these studies were performed.
amounts of IgG and/or IgA demonstrated elevated levels of IgG and IgA FcR+ T cells, respectively, with normal to reduced levels of the other FcR+ T cell subsets. Interestingly, we observed no increases in IgM FcR+ T cells in patients secreting significant amounts of IgM in vitro, perhaps consistent with the fact that there is only one report of a functional IgBF specific for the IgM isotype. All patients tested were hypogammaglobulinemic except CLL242, who had normal serum IgG levels (no IgM or IgA).

**DISCUSSION**

Although numerous studies have described a variety of T cell defects in B-CLL patients, the data we present suggest a direct role for T cells in the modulation of the proliferation and differentiation of the neoplastic B cells. We have begun to elucidate the nature of apparent defects in the T cell-mediated immunoglobulin isotype regulation in B-CLL patients by examining this circuit in a particular patient with abnormal IgA secretion in vitro. Our studies with patient CLL249 indicated that these neoplastic B cells were not intrinsically unresponsive to normal IgA regulatory factors, but rather that the IgABF produced by the host T cells was itself functionally defective. Although the actual amount of IgA secreted in vitro by these tumor B cells was low, this IgA secretion could be suppressed by IgABF produced by normal T cells and not by IgABF produced by the host T cells, and more important, proliferation of the CLL249 B cells was differentially affected in a similar manner by the two factors. The isotype-specific effects of both normal and CLL249 IgABF on proliferation were shown by experiments using IgA- or IgG-producing cell lines. These studies suggest that T cell-mediated class-specific regulatory mechanisms may be importantly altered in B-CLL.

Immunoglobulin binding factors can both suppress and enhance B cell activity in an isotype-specific manner. Work in murine systems suggests that IgBFs are actually heterogeneous mixtures of several factors. Iwashiga determined that potentiating and suppressing activities of rodent IgEBF are produced by the same population of T cells with the factors differing only in degree of glycosylation. Kiyono et al showed the duality of a murine IgABF preparation by using different dilutions, detecting suppression at the higher dilutions and enhancement at the lower dilutions, suggesting the presence of more than one component at differing concentrations. Using gel filtration chromatography, we have shown that normal human IgABF contains at least four subfactors (Fig 3), some of which were decreased or absent in CLL249 IgABF. The differences in activities between normal and CLL249 IgABF suggest that the various suppressing and enhancing effects of IgABF on immunoglobulin synthesis and B cell proliferation may thus be contained in different subfactors, each perhaps affecting a selected group of cells. Further functional and biochemical characterization of each ultraviolet-absorbing peak is currently underway to determine if these are unique or share characteristics with other known growth/regulatory factors.

The disproportionate production of the IgA isotype and the expansion of the IgA-secreting B cell population in CLL249 could occur by a variety of mechanisms related to a loss of a suppressive component of the IgA regulatory circuit that is present in normal IgABF. This could be owing to loss of a subpopulation of T cells that produce the suppressive activity or to altered function of the IgA BF itself. Conversely, an increase in the stimulatory subfactor(s) of IgABF could also lead to the overall enhancement of IgA production.

Whereas the FcR+ T cell-mediated control of B cell function we describe may be important in disease progression, it is yet unclear what role, if any, these T cells have in the development of B-CLL. The variety of T cell defects described in B-CLL patients suggests the possibility that an acquired defect in FcR+ T cells may have led to a change in the suppressing/potentiating balance of immunoglobulin binding factors, contributing to the expansion of a particular B cell clone. The expanding clone could in turn stimulate an increase in the corresponding FcR+ T cell population, although these T cells and their factors are now unable to exert normal controls.

Numerous studies have also shown intrinsic B cell defects to be present in B-CLL. These defects could lead to the proliferation of a clone of B cells of a particular isotype. The abnormal expansion of B cells could indirectly cause a malfunction of the isotype regulatory circuit, providing an abnormal signal to the corresponding FcR+ T cell subset and resulting in a shift of regulatory activity to potentiation. In either case, the fact that the tumor B cells can be regulated by normal immunoglobulin binding factors may have important therapeutic implications. To demonstrate that these sorts of regulatory interactions are not restricted to the control of IgA responses, we are extending our studies to other isotype regulatory circuits. Our initial findings show an association of FcR+ T cells of IgA and IgG specificity with the production of the IgA and IgG isotypes and expansion of the corresponding B cell clone in other B-CLL patients, suggesting the possibility of similar points of regulation. The level of T-γ cells determined to be normal in our studies (Table 1) agrees with that observed by Lum et al., but is lower than that reported by other investigators. We are currently developing a method for measuring FcR+ T cells by flow cytometry to eliminate the need for cumbersome and perhaps less sensitive rosetting procedures. In addition, studies of other classes of Ig binding factors and their role in B-CLL are currently underway.

Hypogammaglobulinemia is an important pathologic consideration in B-CLL, since infections are a major cause of morbidity and mortality. A possible role for T cells in inducing hypogammaglobulinemia has been suggested in other studies. Although it is difficult to derive meaningful conclusions concerning disease progression in vivo, the studies we present suggest that therapeutic manipulation of the immunoglobulin isotype regulatory circuit by the heterogeneous group of immunoglobulin binding factors could influence the hypogammaglobulinemia as well as the proliferation of the neoplastic clone in B-CLL.

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REFERENCES

6. Kay NE: Abnormal T cell subpopulation function in CLL: Excessive suppressor (T gamma) and deficient helper (T mu) activity with respect to B cell proliferation. Blood 57:418, 1981
31. Katz DH: The IgE antibody system is coordinately regulated by FcR epsilon-positive lymphoid cells and IgE selective soluble factors. Int Arch Allergy Appl Immunol 77:21, 1985
39. Moretta L, Ferrarini M, Durante ML, Mingari MC: Express-
42. Cillari E, Domenico L, Salerno A, LaVia M: Enumeration of T0 lymphocytes in B-chronic lymphocytic leukemia (B-CLL) by a rapid double-rosetting technique. Diag Immunol 2:224, 1984
43. Foon KA, Schroff RW, Gale RP: Surface markers on leukemia and lymphoma cells: Recent advances. Blood 60:1, 1982
44. Revillard J-P, Millet I, Vincent C: A non-isotype specific suppressor factor contaminates preparations of constitutive human IgG- or IgA-binding factors. Proceedings of the Sixth International Congress of Immunology (abstr 2.63.18)
Defective T cell-mediated, isotype-specific immunoglobulin regulation in B cell chronic lymphocytic leukemia

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