Idiotypic Cross-Reactivity of Immunoglobulins Expressed in Waldenström’s Macroglobulinemia, Chronic Lymphocytic Leukemia, and Mantle Zone Lymphocytes of Secondary B-Cell Follicles

By Ofra Axelrod, Gregg J. Silverman, Vip Dev, Robert Kyle, Dennis A. Carson, and Thomas J. Kipps

Monoclonal antibodies (MoAbs) specific for autoantibody-associated cross-reactive idiotypes (CRIs) of Waldenström’s IgM react frequently with the surface Ig (sIg) expressed by leukemia cells of patients with chronic lymphocytic leukemia (CLL). Evaluation of the molecular basis for this cross-reactivity indicates that such CRIs are encoded by conserved antibody variable region genes (V genes) that have undergone little or no somatic hypermutation. We find that such anti-CRI MoAbs stain a subpopulation of cells within the mantle zones surrounding the germinal centers of normal human tonsil. In contrast, MoAbs specific for variable region subgroup determinants react with cells in both the mantle zones and germinal centers of secondary B-cell follicles. To test whether mantle zone B cells not reactive with existing anti-CRI MoAbs may express sIg bearing as-yet-unrecognized CRIs present on sIgs produced by neoplastic cells of some patients with Waldenström’s macroglobulinemia or CLL, we immunized mice with purified Waldenström’s IgM that have been characterized for their variable region subgroup.

The cross-reactive idiotypes (CRIs) of human Ig (hu-Ig) can be useful serologic markers for expression of hu-Ig variable region genes (V genes). Two of the best characterized human CRIs to date are defined by reactivity with monoclonal antibodies (MoAbs), designated 17.109 and G6. These MoAbs were generated against CRIs present on Waldenström’s hu-IgM paraproteins with anti-IgG or rheumatoid factor (RF) binding activity.1,2 Subsequently, they were shown to react frequently with the leukemia cells from unrelated patients with B-cell chronic lymphocytic leukemia (B-CLL) and related B-cell lymphomas,3-5 and virgin B cells within the primary follicles of human fetal spleen.6 Investigations of the genetic basis for expression of these CRIs show that each is encoded by a highly conserved Ig variable region gene(s) (V gene) present in the germline DNA.7,8 As such, these CRIs apparently are serologic markers for expression of these V genes without substantial somatic mutation.

Waldenström’s IgM macroglobulins may be particularly well suited to serve as immunogens for the generation of additional MoAbs specific for the protein products of conserved hu-Ig V genes. Although “rescued” human lymphoma antibody or myeloma paraproteins of unknown specificity have been used to induce murine anti-CRI Ig-producing cells,11-13 the V genes encoding such antibodies may harbor numerous somatic mutations.14 These mutations may disrupt determinants encoded by V genes present in the germline DNA. At least a subgroup of Waldenström’s tumors express hu-IgM proteins encoded by V genes that apparently have not undergone substantial somatic mutation, however.1 Thus, immunization of mice with such hu-IgM proteins may induce antibodies that, like 17.109 or G6, can serve as useful serologic probes for expression of conserved V genes.

We describe the production and characterization of anti-CRI MoAbs against Waldenström’s hu-IgM paraproteins by a novel screening method. MoAbs directed against major CRIs on hu-Ig stain a subset of lymphocytes within the mantle zone surrounding the germinal centers of human tonsil. Based on this observation, we screened our hybridoma supernatants for their ability to stain tonsilar B lymphocytes in fresh-frozen tissue sections. Hybridomas producing antibodies that react with tissue sections in a fashion similar to that of other known anti-CRI MoAbs were identified and isolated. We describe the identification and characterization of two such hybridomas.

MATERIALS AND METHODS

Antibodies. An IgG, MoAb specific for a V<sub>4</sub>H<sub>11b</sub> subgroup determinant(s),9 was obtained from Dr George Abraham (University of Rochester, Rochester, NY). B6, a murine IgG, MoAb generated against the heavy chain of an IgM-RF paraprotein and reactive with a V<sub>3</sub>subgroup-associated CRI,10 and G6, a murine IgG, MoAb,1 were provided by Drs Rizgar Mageed and Roy Jefferis (University of Birmingham, Birmingham, England). MoAb 6B6, specific for a V<sub>3</sub>a-associated CRI,10 was provided by Drs Ralph E. Schroenkleber and William J. Koopman (University of...
or A light chain-producing hybridomas were as described previously.10 Alabama, Birmingham, AL). MoAb 17.109 is as described previously.11 Mouse MoAb was purified from ascites by ammonium sulfate precipitation and either absorption with QAE (Pharmacia Fine Chemicals, Upsala, Sweden) (for IgG, and IgG3) or protein A Sepharose-column chromatography (BioRad, Richmond, CA). hu-IgM paraproteins (Table 1) either were isolated from sera of patients with Waldenström’s macroglobulinemia by 45% saturated ammonium sulfate precipitation and Sephadex-G200 (Pharmacia) column chromatography or were obtained from commercial sources (eg, Caltag, San Francisco, CA; Jackson ImmunoResearch Laboratories, West Grove, PA; Binding Site, Birmingham, England; or Tago, Burlingame, CA). Igλ light chain Bence Jones proteins were provided by Dr A. Solomon (Department of Medicine, University of Tennessee, Knoxville, TN). IgM proteins A224 and L16 (Table 2), which have known heavy chain variable regions of Vλ5 and Vδ6, respectively, were provided by Dr Ton Logtenberg (Academisch Ziekenhaus, Utrecht, The Netherlands). The human paraproteins used were analyzed by polyacrylamide gel electrophoresis (PAGE) and immunoblotting with antisynthetic peptide antisera, each specific for a primary structural determinant(s) of a given hu-Ig variable region subgroup, as described previously.10 Daudi, a Burkitt’s lymphoma cell line producing κ light chains of the Vκ1 subgroup,21 was grown in serum-free tissue culture medium (HL-1, Ventrex Laboratories, Portland, ME). hu-Ig produced by this cell line was purified by precipitation of culture supernatants with 45% ammonium sulfate. To isolate the κ light chain of the IgMκ paraprotein MAR (Table 1) we reduced the purified IgM in 10 mmol/L dithiothreitol (Calbiochem, La Jolla, CA) at 37°C for 2 hours in 0.5 mol/L Tris HCl, 2 mmol/L EDTA (pH 7.6) before adding iodoacetamide (Sigma Chemical, St Louis, MO) to a final concentration of 25 mmol/L. After a 60-minute incubation at 4°C, the antibody heavy and light chains were separated on an AcA34 column (LKB, Upsala, Sweden) equilibrated with 3 mol/L guanidine HCl and 0.25 mol/L ammonium bicarbonate at pH 8.2.

Separation of heavy and light chains was achieved by electrophoresis in a 3% stacking gel and a 5% separating gel using Tris-glycine buffer (pH 8.3). The gel was stained by incubation with Coomassie blue R-250 for 2 hours and then destained in 10% acetic acid, rinsed in water, and dried. The heavy chain, which migrates to a position lower than the light chain, was excised and eluted from the gel with 0.1 mol/L acetic acid. The antigenic specificity of each component was tested by immunoblotting with antisynthetic peptide antisera, each specific for a primary structural determinant(s) of a given hu-Ig variable region subgroup.10

Table 1. Panel of hu-IgM Paraproteins

<table>
<thead>
<tr>
<th>No.</th>
<th>MoAb Designation</th>
<th>Light Chain</th>
<th>Heavy Chain</th>
<th>CRI</th>
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<tr>
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<td>IgMκ</td>
<td>Vλ3</td>
<td>Vκ4</td>
<td>17.109</td>
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The heavy and light chain subgroups determined for each paraprotein are shown. Proteins 1 through 5 were obtained from the Binding Site (Birmingham, England), Calbiochem (La Jolla, CA), Caltag Laboratories (San Francisco, CA), Jackson ImmunoResearch Laboratories (West Grove, PA), and Tago (Burlingame, CA), respectively. Paraproteins 6 through 8 and 9 through 15 were purified from serum samples of patients with Waldenström’s macroglobulinemia at the Scripps Clinic (La Jolla, CA) and Mayo Clinic (Rochester, MN), respectively.

Table 2. VOH3 Binding to Representative Paraproteins With Heavy Chains of Different Vκ Subgroups

<table>
<thead>
<tr>
<th>No.</th>
<th>Designation</th>
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<th>Heavy Chain</th>
<th>CRI* to VOH3</th>
<th>CRI to Anti-Cp</th>
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<td>190</td>
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<td>Vλ3</td>
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<td>Vκ3</td>
<td>Vλ3</td>
<td>NR†</td>
<td>200</td>
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**CRI** is the concentration (in ng/mL) of hu-Ig required to achieve 50% of maximum binding to either VOH3 or an anti-Cp MoAb.

†NT indicates that the protein was nonreactive with VOH3 at an excess saturating concentration (5 μg/mL), and therefore was not titrated on plates coated with anti-CRI or anti-constant region MoAb.

‡NR indicates that the protein was nonreactive at the highest concentration tested.

*Generation of monoclonal anti-idiotypes. Fν1 (BALB/c × A/J) mice (Research Institute of Scripps Clinic, La Jolla, CA) were immunized with 100 μg purified paraprotein emulsified in complete Freund’s adjuvant (Sigma) administered subcutaneously (SC). After 3 weeks, these animals received two subsequent booster injections of 100 μg purified paraprotein emulsified in incomplete Freund’s adjuvant (Sigma) at intervals more than 1 week apart. Three days before fusion, mice were boosted intraperitoneally (IP) with 100 μg paraprotein dissolved in phosphate-buffered saline (PBS, pH 7.2). Fusion was performed as described,27 with slight modifications. A non-Ig-producing variant of P3-X-63-Aby8 resistant to 10−4 mol/L 8-azaguanine was fused with mouse spleen cells using polyethylene glycol (PEG) 1500 (Boehringer Mannheim Biochemicals, Indianapolis, IN). Cells were suspended in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 15% fetal calf serum (FCS) (HyClone, Logan, UT) and 10% hybridoma growth factor (IGEN, Rockville, MD) and seeded at 1 × 105/mL in 24-well plates. Twenty-four hours later, selection medium (supplemented with hypoxanthine-aminopterin-thymidine, Boehringer Mannheim) was added to cultures. Ten to 14 days later, supernatants were collected and checked for reactivity. Hybridoma cultures corresponding to positive supernatants were subcloned by limiting dilution. Positive subclones were injected IP into pristane-primed syngeneic mice to produce ascites.

*Enzyme-linked immunoabsorbent assay (ELISA). Polystyrene microtiter plates were coated with hu-IgM at 5 μg/mL in borate-buffered saline (BBS), pH 8.2. Plates were washed free of unbound material with borate buffer (BBS at pH 8.2) containing 1% bovine serum albumin (BSA) to saturate residual plate protein binding sites. Serum or supernatant samples diluted in 1% BSA in BBS were added to the plates and allowed to incubate overnight. Plates then were washed with 0.05% Tween detergent in BSA/BBS before incubation with alkaline-phosphatase-conjugated heterologous antibody specific for mouse Ig (Southern Biotech, Birmingham, AL). After a subsequent 1-hour incubation,
the plates were washed with BSA/BBS, developed with freshly prepared p-nitrophenyl phosphate disodium (Sigma) in carbonate buffer, and then monitored at OD$_{405}$ with an ELISA plate reader. hu-IgM with variable region subgroups distinct from that of the hu-IgM immunogen was added to hybridoma supernatants to a final concentration of 100 µg/mL. After an overnight incubation at 4°C, these supernatants were reexamined for reactivity with the hu-IgM immunogen or an irrelevant hu-IgM.

**Immunohistochemistry.** Residual lymphoid tissue from tonsillectomies were frozen in optimum cutting temperature medium (Miles Laboratories, Naperville, IL). Four-micron sections were prepared from the tissue blocks of frozen human tonsil for immunohistochemical analyses with an avidin-biotin complex immunoperoxidase technique, as described previously.8 Hybridoma supernatants with and without exogenous hu-IgM (at 10 µg/mL) were incubated overnight at 4°C before use. Sixty microlitres culture supernatant was added to each slide and allowed to incubate at room temperature for 60 minutes. Slides were washed with PBS (pH 7.2) before 60 µl biotinylated horse anti-mouse Ig 3.3 µg/mL was added (Vector Laboratories, Burlingame, CA). After another 60-minute incubation at room temperature, the slides were washed and then exposed to avidin D coupled to horse radish peroxidase (Vector Laboratories) for 60 minutes at 10 µg/mL. After washing the slides with PBS, we developed the bound peroxidase with 3-amino-9-ethylcarbazole (Sigma) at 0.4 mg/mL in 0.015% hydrogen peroxide (Sigma) in 0.1 mol/L sodium acetate (pH 5.2) for approximately 15 minutes. Washed slides then were stained with Mayer’s haematoxylin (Sigma) for 2 minutes before being mounted.

**Immunoblot analysis.** hu-Ig were analyzed for variable region subgroup or for reactivity with identified anti-CRI MoAb using immunoblotting as described previously. Test paraproteins were run on polyacrylamide gel under denaturing and reducing conditions. Thereafter, separated heavy and light chains were transferred electrothermietically to a transfer membrane (Immobilon-P, Millipore) and incubated with either anti-CRI MoAb, anti-heavy or antilight chain antibody (mouse anti-IgM or goat anti-human κ light chain, Calbiochem) or rabbit antisera generated against peptides corresponding to variable region subgroups as described previously. Filters were developed with $^{125}$I-protein A (ICN Biomedicals, Costa Mesa, CA).

**Flow cytometry.** Leukemia cells from patients with CLL were as described previously.9 Indirect immunofluorescence analyses of the slg expressed by leukemia cells of patients with CLL with anti-CRI MoAbs were performed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Leukemia cells were incubated in staining medium (SM, consisting of RPMI 1640, 3% FCS, 100 mmol/L HEPES (pH 7.6) and 1 µg/mL propidium iodide) containing saturating amounts of anti-CRI MoAb or an irrelevant mouse MoAb of the same isotype. After 20 minutes at 4°C, cells were washed in propidium-iodide-deficient SM and then developed with fluorescein-conjugated goat antibodies specific for mouse Ig (Southern Biotechnology Associates, Birmingham, AL). Dead cells with fluorescence at greater than 600 nm when excited at 488 nm were excluded from the analyses.

**RESULTS**

**Characterization of hu-IgM paraproteins.** By immunoblotting with heterologous antisera generated against synthetic peptides corresponding to subgroup-specific residues, we determined the variable region subgroups of the heavy and light chains of each of the assembled Waldenström’s hu-IgM paraproteins (Table 1) and Bence Jones proteins. hu-IgM heavy chains reacted with only one of the six different antisera specific for each of the heavy chain subgroups, allowing us to assign a single heavy chain subgroup for each hu-Ig. Ten hu-IgM (67%) belonged to the V$_{H}$3 subgroup, four (27%) belonged to the V$_{H}$4 subgroup, and only one (7%) belonged to the V$_{H}$6 subgroup. Of the light chain variable regions of the 11 hu-IgM that had κ light chains, five (45%) belonged to the V$_{K}$1 subgroup, three (27%) represented the V$_{K}$3 subgroup, two (18%) were V$_{K}$2, and only one belonged to the V$_{K}$4 subgroup (Table 1). Similarly, we could assign the light chains of κ hu-Ig to one of the four κ light chain subgroups. Of the 18 κ light chain Bence Jones proteins available, 10 (55%) were assigned to V$_{K}$1, three (17%) to V$_{K}$2, three (17%) to V$_{K}$3, and two (11%) to V$_{K}$4. The variable region subgroups of λ light chains were not determined.

In addition, we tested the hu-IgM paraproteins for reactivity with each of several existing anti-CRI MoAbs (Table 1). Three of the 10 paraproteins characterized as having heavy chain variable regions of the V$_{H}$3 subgroup reacted with B6, consistent with recognition of a V$_{H}$3-associated CRI by this MoAb.19 Two of the three paraproteins with κ light chains of the V$_{K}$3 subgroup reacted with 17.109. The other paraprotein with V$_{K}$3 light chains was recognized by 6B6.6. To produce MoAbs reactive with previously undetected CRIls, we chose to generate MoAbs against MAR (a V$_{K}$1V$_{H}$1 hu-IgM, Table 1) and ME591 (a V$_{K}$V$_{H}$3 hu-IgM, Table 1). Unlike other Waldenström's hu-IgMs previously used to generate anti-CRI MoAbs, these two paraproteins had neither RF activity nor antinuclear autoantibody activity (data not shown).

**ELISA screen for anti-idiotypic MoAbs.** Ten to 14 days after fusion, supernatants from growing hybridoma cultures were assayed for anti-idiotypic activity in an ELISA. Supernatants were incubated on ELISA plates coated with the hu-IgM immunogen. Because the hybridomas of each well may be oligoclonal, hu-IgM of irrelevant heavy and light chain variable region subgroups was added to the positive supernatants to absorb possible activity against the constant portion of the hu-IgM antibody molecule (Table 1). Supernatants with reactivity for the hu-IgM immunogen in the presence of an irrelevant hu-IgM were scored as having potential antiidiotypic activity.

**Immunohistochemical detection of MoAbs specific for human CRI.** Anti-CRI MoAbs can be detected by screening hybridoma supernatants on tissue sections of human tonsil. Antibodies specific for a major CRI stain a subpopulation of tonsilar lymphocytes that have a distinctive histologic distribution; eg, 17.109, specific for a κ light chain-associated CRI encoded by a conserved V$_{H}$3 gene, labels a subpopulation of lymphocytes that reside in the mantle zone surrounding each of the germinal centers (Fig 1A). This distinctive distribution of CRI-reactive cells has been noted in sections of every tonsil specimen examined to date (n > 24). In contrast, MoAbs directed against variable region subgroup determinants stain a subpopulation of cells in both the mantle zones and germinal centers (Fig 1B).

Supernatants with potential antiidiotypic reactivity by
ELISA were used to stain sections of fresh-frozen human tonsil. Supernatants were tested with and without an excess concentration (100 μg/mL) of the hu-IgM immunogen or irrelevant hu-IgM protein. A few wells from each fusion reacted with a subpopulation of cells within the mantle zone in the presence of added irrelevant hu-IgM protein (data not shown). Addition of the original hu-IgM immunogen to such supernatants completely blocked their reactivity for human tonsil (data not shown). From such tissue reactivity, these supernatants were assumed to have anti-CRI activity.

Hybridomas with supernatants having presumed anti-CRI activity were cloned by limiting dilution. Two MoAbs produced in this way, OAK1 [specific for a CRI present on MAR (Table 1)] and VOH3 [specific for a CRI present on ME591 (Table 1)], retained the ability to stain a subpopulation of lymphocytes confined to the mantle zones of the secondary follicles of human tonsil (eg, Fig 1D).

Specificity and cross-reactivity of OAK1 and VOH3. The reactivity of OAK1 was assayed against each of the 15 characterized Waldenström's IgM proteins (Table 1), 18 κ light chain Bence Jones proteins, and the separated heavy and light chains of MAR, the hu-IgM paraprotein used to induce OAK1. Each protein was screened at 5 μg/mL in an ELISA using plates precoated with anti-CRI MoAb. In addition, to compare the relative binding intensities of the anti-CRI for the various Ig proteins, representative hu-Ig were each titrated in parallel on plates precoated with anti-CRI or anti-constant region MoAb. From these studies, we found that OAK1 binds to the isolated κ light chain of MAR, the immunogen used to induce OAK1 (Table 3). Furthermore, MoAb bound to several hu-Ig with κ light chains of the VK1 variable region subgroup, but did not bind to the isolated heavy chain of MAR or to any hu-Ig with either λ light chains or κ light chains belonging to the Vκ2, Vκ3, or Vκ4 subgroup (Tables 1 and 3). Titration of OAK1-reactive hu-Ig showed each to have comparable binding activities to plates coated with either OAK1 or an anti-κ constant region MoAb (Table 3), indicating that the relative binding activities of OAK1 for each of the OAK1-reactive hu-Ig are similar. OAK1 did not react with all hu-Ig with κ light chains of the Vκ1 subgroup, however; eg, although the actual or deduced amino acid sequences of AU, HAU, SCW, and DAUDI place these κ proteins in the VK1 subgroup, these proteins are not bound by the OAK1 MoAb (Table 3). In all, OAK1 recognizes 5 of 15 (33%) of the tested hu-Ig with κ light chains of the Vκ1 subgroup. From these data, we conclude that OAK1 is specific for a Vκ1-associated CRI rather than a Vκ1-subgroup determinant(s).

Immunoblotting confirmed that OAK1 is specific for a CRI present on the κ light chains belonging to the Vκ1 variable region subgroup (Fig 2). OAK1 reacted with the separated κ light chains of the hu-Ig immunogen. Furthermore, OAK1 exclusively bound the separated κ light chains of the Vκ1, Vκ3, or Vκ4 subgroup (Tables 1 and 3). Titration of OAK1-reactive hu-Ig showed each to have comparable binding activities to plates coated with either OAK1 or an anti-κ constant region MoAb (Table 3), indicating that the relative binding activities of OAK1 for each of the OAK1-reactive hu-Ig are similar. OAK1 did not react with all hu-Ig with κ light chains of the Vκ1 subgroup, however; eg, although the actual or deduced amino acid sequences of AU, HAU, SCW, and DAUDI place these κ proteins in the VK1 subgroup, these proteins are not bound by the OAK1 MoAb (Table 3). In all, OAK1 recognizes 5 of 15 (33%) of the tested hu-Ig with κ light chains of the Vκ1 subgroup. From these data, we conclude that OAK1 is specific for a Vκ1-associated CRI rather than a Vκ1-subgroup determinant(s).

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Immunoblotting confirmed that OAK1 is specific for a CRI present on the κ light chains belonging to the Vκ1 variable region subgroup (Fig 2). OAK1 reacted with the separated κ light chains of the hu-Ig immunogen. Furthermore, OAK1 exclusively bound the separated κ light chains of the Vκ1, Vκ3, or Vκ4 subgroup (Tables 1 and 3). Titration of OAK1-reactive hu-Ig showed each to have comparable binding activities to plates coated with either OAK1 or an anti-κ constant region MoAb (Table 3), indicating that the relative binding activities of OAK1 for each of the OAK1-reactive hu-Ig are similar. OAK1 did not react with all hu-Ig with κ light chains of the Vκ1 subgroup, however; eg, although the actual or deduced amino acid sequences of AU, HAU, SCW, and DAUDI place these κ proteins in the VK1 subgroup, these proteins are not bound by the OAK1 MoAb (Table 3). In all, OAK1 recognizes 5 of 15 (33%) of the tested hu-Ig with κ light chains of the Vκ1 subgroup. From these data, we conclude that OAK1 is specific for a Vκ1-associated CRI rather than a Vκ1-subgroup determinant(s).
of some but not all hu-Ig with \( \kappa \) light chains of the \( \text{V}_{\kappa} \) variable region subgroup (Fig 2).

VOH3, on the other hand, binds an Ig heavy chain-associated CRI. Similar analyses with VOH3 showed that this anti-CRI has binding activities for hu-IgM paraproteins, A1887 or WH951, that are comparable to that for the hu-IgM protein, ME591, used to generate this MoAb (Table 2). All three of these Waldenström’s paraproteins have heavy chain variable regions belonging to the \( \text{V}_{\kappa} \) subgroup. Immunoblotting demonstrated that VOH3 binds to the separated heavy chains of VOH3-reactive paraproteins (Fig 3). However, VOH3 did not bind to any of the other 12 Waldenström’s IgM paraproteins (Table 1) or to representative IgM proteins of all six \( \text{V}_{\kappa} \) subgroups other than \( \text{V}_{\kappa} \) (Table 3). Moreover, VOH3 did not react with all hu-Ig of the \( \text{V}_{\kappa} \) subgroup; eg, VOH3 did not react with any of the hu-Ig found positive for B6, a CRI associated with Ig heavy chain variable regions of the \( \text{V}_{\kappa} \) subgroup (Tables 1 and 2 and Fig 3). In addition, although the separated heavy chains of either serum IgM or IgG react with the antipeptide antisera specific for Ig \( \text{V}_{\kappa} \) subgroup (Fig 3, group B, lanes 7 and 8, respectively), they do not have detectable reactivity for VOH3 by immunoblot analysis (Fig 3, group A, lanes 7 and 8, respectively). Collectively, these studies indicate that VOH3 is specific for a CRI that is distinct from B6 and present on a subset of heavy chain variable regions of the \( \text{V}_{\kappa} \) subgroup.

Reactivity of OAK1 and VOH3 with lymphocytes of CLL patients. The newly generated anti-CRI MoAb reacted with the leukemia cells of unrelated patients with CLL. By flow-cytometric analysis, OAK1 was shown to stain the leukemia cells from 5 of 20 patients (25%) with \( \kappa \) light chain-expressing CLL (eg, Fig 4). In this group of 25 patients, three had leukemia cells reactive with the 17.109-CRI. Consistent with OAK1 recognizing a \( \text{V}_{\kappa} \)-associated CRI, none of the three 17.109-reactive CLL expressed the OAK1 CRI. In addition, none of the leukemia cells from patients with \( \lambda \) light chain-expressing CLL reacted with OAK1. Despite recognizing a CRI associated with heavy chain variable regions of the relatively large \( \text{V}_{\kappa} \) subgroup, however, VOH3 reacted with the leukemia lymphocytes from only 1 of 17 patients tested (data not shown). Again, the leukemia cells reactive with VOH3 did not express the B6-CRI.

**DISCUSSION**

MoAbs generated against Waldenström’s macroglobulins specific for prominent CRIs expressed in CLL stain a subpopulation of lymphocytes within human tonsil. The tonsillar B cells expressing these CRIs are confined to the mantle zones surrounding the germinal centers of secondary B-cell follicles. Taking advantage of this anatomic distribution of CRI-reactive B cells, we developed methods to screen for additional anti-CRI/MoAb-producing hybri- domas. This allowed us to identify two new anti-CRI/MoAbs, designated OAK1 and VOH3, that bind exclusively to a subset of mantle zone lymphocytes. Immunohistochemical studies demonstrated that these MoAbs recognize a CRI determinant(s) present on a subset of \( \kappa \) light chains of the \( \text{V}_{\kappa} \) subgroup or a subset of Ig heavy chains of the \( \text{V}_{\kappa} \) subgroup, respectively. Including the hu-IgM used as immunogen, OAK1 or VOH3 reacted with 27% (4 of 15) or 20% (3 of 15) of the hu-IgM paraproteins assembled for this study, respectively (Table 1), suggesting that these CRIs are frequently present on the IgM paraproteins of patients with Waldenström’s macroglobulinemia. Furthermore, a screening of unrelated patients with CLL suggests that the OAK1 and VOH3 CRIs also may be expressed by the leukemia cells in this disease. The overall success of these methods indicates that variable regions of the Igs produced by mantle zone B cells share idiotypic determinants with Igs expressed in Waldenström’s macroglobulinemia and B-CLL. Certainly the expression frequencies of these CRIs may differ among patients with Waldenström’s macroglobulinemia or CLL. In a previous study of IgM paraproteins mostly from patients with Waldenström’s macroglobulinemia, 10 of 23 (43%) were typed as having heavy chain variable
regions that were most likely encoded by VN genes of the V3 subgroup, the largest of the VN gene subgroups in the human heavy chain locus. Of the 15 Waldenström's proteins typed in these studies, two thirds had heavy chain variable regions of the V3 subgroup (Table 1). Although VN genes belonging to the V3 gene family may encode polyreactive antibodies produced by CD5 B cells, antibodies of the V3 subgroup may be proportionately underrepresented among the IgGs expressed in CLL.\(^{4-6,8-10,12}\) Consistent with this notion, VOH3, although reactive with 30% (20%) of the tested Waldenström's proteins, reacted only with the leukemia cells from 1 of 17 (6%) randomly selected patients with CLL. This may reflect a difference in VN gene subgroup use between these two B-cell malignancies.

In addition, the results of our limited survey suggest that the expression frequencies of these CRIs may differ between patients with Waldenström's macroglobulinemia or multiple myeloma. Although the proportion of k light chain Waldenström paraproteins typed as V3 (45%) was comparable with that of V3 k Bence Jones paraproteins (55%), OAK1 reacted with 80% (4 of 5) of the Waldenström's hu-Ig with k light chains of the V3 subgroup, but only 10% (1 of 10) of the V3 myeloma light chains. Conceivably, the VN genes encoding many of these VN Bence Jones proteins may have incurred somatic mutations, ultimately disrupting the CRI determinant(s) recognized by OAK1. Alternatively, although not excluding the former notion, the VN genes expressed in multiple myeloma may differ from those used in Waldenström's macroglobulinemia.

Differences in the expression frequencies of CRIs in B-cell malignancies may reflect apparent differences in the CRI expression frequencies of various subpopulations of normal B cells. Each of these anti-CRI MoAbs stains a subpopulation of mantle zone lymphocytes but rarely any germinal center B cells. Conceivably, germinal center B cells may express V genes that differ from those expressed by mantle zone B cells. Alternatively, although not excluding the former hypothesis, germinal center B cell may express V genes that have undergone somatic hypermutation. In this regard, the scarcity of CRI+ cells in the germinal centers may mimic the low frequencies at which such CRIs are detected on Ig expressed by non-Hodgkin's lymphomas of presumed follicular center cell origin. The Ig V genes expressed by such lymphomas demonstrate intraclonal diversity in their expressed Ig V genes indicative of ongoing somatic mutation.\(^{11}\) Somatic mutation of the V genes expressed by these lymphomas, and the germinal center cells from which they are derived, may disrupt and destroy variable region determinants that are recognized by certain anti-CRI MoAbs. Consistent with this notion, MoAbs specific for subgroup determinants that are more resilient to the structural permutations induced by somatic hypermutation stain cells in both the germinal centers and the surrounding mantle zones.

The use of typed Waldenström's IgM paraproteins together with the screening methods we describe may facilitate detection of additional MoAbs specific for important but as yet unidentified CRIs. Previous methods required testing anti-idiotypic antibodies on a panel of purified hu-Ig or assaying for inhibition of anti-idiotyp/idiotype binding with pooled normal hu-IgM or plasma.\(^{11-13}\) In the former method, the panel of hu-Ig had to be large enough to detect less common CRIs. Using the latter method, less common CRIs or CRIs not present on secreted hu-Ig may not be detected. By analyzing the staining pattern of each hybridoma culture supernatant on human tonsil, however, we can distinguish between MoAbs having antiprivate idotype, anti-CRI, antisubgroup, or anticonstant region binding activity. As we have shown, newly generated anti-CRI MoAbs may react with leukemia cells from patients with CLL. Batteries of MoAbs recognizing disparate CRIs may allow early detection and possible immunotherapy of B-cell neoplasia in a large proportion of patients.\(^{11,13,33-35}\) Finally, as the molecular basis for idiotypic cross-reactivity is resolved, such reagents may be useful for probing V gene expression in B-cell lymphoproliferative, immunodeficiency, or autoimmune diseases.

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Idiotypic cross-reactivity of immunoglobulins expressed in Waldenstrom's macroglobulinemia, chronic lymphocytic leukemia, and mantle zone lymphocytes of secondary B-cell follicles

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