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Immunocytochemical Detection of Kappa and Lambda Light Chain V Region Subgroups in Human B-cell Malignancies

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We have used a sensitive immunoperoxidase method and highly specific anti-light chain antisera to determine the light chain variable region (V_L) subgroup nature of cytoplasmic (c) and cell surface (s) Ig expressed by human monoclonal plasma cells and B lymphocytes. The immunocytochemical characterization of cIg and sIg used antisera specific for the established κ light chain $V_κ$ subgroups ($V_{κI}$, $V_{κII}$, $V_{κIII}$, and $V_{κIV}$) and the λ light chain $V_λ$ subgroups ($V_{λI}$, $V_{λII/V}$, $V_{λIII}$, $V_{λIV}$, and $V_{λVI}$). Studies were performed using cytospin preparations of bone marrow-, peripheral blood-, and lymph node-derived cells from patients with multiple myeloma, amyloidosis AL, and Waldenström's macroglobulinemia and with low-, mid-, and high-grade B-cell malignancies. The $V_κ$ or $V_λ$ subgroup of the cIg or sIg also could be identified after deparaffinization and enzyme treatment of formalin-fixed, paraffin-embedded specimens. For those patients who had monoclonal serum or urinary Igs, there was complete concordance between the V_L subgroup of the secreted Ig and that of the cIg or sIg. The percentage distribution of $V_κ$ or $V_λ$ subgroups on the sIg of cells from patients with chronic lymphocytic leukemia (CLL) and other cytomorphologic types of B-cell malignancies differed from that found for κ- or λ-type Bence Jones proteins obtained from patients with multiple myeloma, amyloidosis AL, and Waldenström's macroglobulinemia. In contrast to the plasma cell and lymphocytoid plasma cell diseases, a relative predominance of certain V_L subgroups, ie, $V_{κIV}$, $V_{λIII}$, and $V_{λIV}$, and the absence of the amyloid-associated $V_{λVI}$ subgroup were found in CLL and related diseases. The immunocytochemical techniques used make possible a rapid means to demonstrate B-cell monoclonality and provide further evidence for the selective expression of certain V_L genes in human B-cell neoplasia. (Am J Pathol 1990, 137:855–862)

B-cell–related neoplasms represent monoclonal proliferative diseases that are distinguished by distinctive immunoglobulin (Ig) phenotypic properties. The clonal nature of these disorders is evidenced by the expression of Ig genes and their monoclonal protein products. One such product, common to all classes of Ig, is the kappa (κ) or lambda (λ) light polypeptide chain. Human κ and λ light chains can be classified into multiple variable region (V_L) subgroups on the basis of characteristic amino acid and nucleic acid sequence homologies.[1–3] Such subgroups have also been differentiated serologically.[4,5] Antisera, prepared against monoclonal light chains, can recognize each of the chemically defined $V_κ$ and $V_λ$ subgroups, designated $V_{κI}$, $V_{κII}$, $V_{κIII}$, $V_{κIV}$ and $V_{λI}$, $V_{λII/V}$, $V_{λIII}$, $V_{λIV}$, $V_{λVI}$ (proteins classified chemically as $V_{λV}$ react with specific anti-$V_{λII}$ antisera). In addition, multiple V_L sub-subgroups have been demonstrated serologically,[5,6] and these presumably (like the V_L subgroups) represent the products of single or closely related V_L genes. A functional role for V_L subgroups (and sub-subgroups) is shown by the association of particular V_L genes with certain types of autoantibodies,[7–13] amyloidosis AL,[14] and B-cell neoplasms.[15–18]

To study further the distribution and importance of V_L subgroups as biologic markers of B-cell lymphoproliferative disorders, we have used highly specific anti-light chain antisera prepared in our laboratory to analyze immunocytochemically the cellular Ig of monoclonal B-cell–related populations found in patients with multiple myeloma, Waldenström's macroglobulinemia, amyloidosis AL, chronic lymphocytic leukemia (CLL), and other forms of malignant lymphoma. These studies have demonstrated the capability of such antisera to identify the V_L

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subgroup nature of cytoplasmic (c) and cell-surface (s) Ig in bone marrow-, peripheral blood-, and lymph node-derived cytospin preparations and in tissue sections. The ability to demonstrate immunocytochemically the V_κ or V_λ subgroup provides a unique diagnostic tool for the establishment of B-cell monoclonality, as well as a rapid and effective means of determining V_L gene usage in human B-cell neoplasia.

Methods and Materials

Anti-light Chain Antisera

The methods used in the preparation and characterization of our polyclonal anti-human κ or λ light chain and V_L subgroup-specific antisera have been described previously.[4] Briefly, albino New Zealand rabbits were immunized with κ or λ Bence Jones proteins or V_L-related fragments and the reactivity of the antisera was determined through comparative immunodiffusion analyses using completely sequenced light chains representative of the human V_κ and V_λ subgroups. The antisera were rendered specific for κ or λ light chain or V_L subgroup-associated determinants, as established by ELISA and immunoblotting analyses, through extensive absorption with appropriate heterologous light chains and non-Ig proteins.

Immunophenotyping

Specimens containing mononuclear cells obtained from bone marrow, peripheral blood, or teased from lymph nodes were enriched by ficoll density gradient (Histopaque-1077, Sigma Diagnostics, St. Louis, MO) and prepared by centrifugation onto clean glass slides using a Shandon cytocentrifuge (Shandon, Inc., Pittsburgh, PA). The slides were fixed for 30 minutes in acetone, air-dried overnight, and washed extensively in a 0.25-mol/l (molar) sodium phosphate, 0.15 mol/l sodium chloride (PBS) buffer, pH 7.2. For intact fresh tissue specimens, 4-μm sections were cut in a cryostat, placed on 0.5% gelatin-coated slides, and dried overnight. They were fixed and washed as previously stated. Immunostaining was performed using the avidin-biotin complex (ABC) method (Vectastain Peroxidase ABC kit, Vector Laboratories, Inc., Burlingame, CA) and serial dilutions of our primary rabbit anti-human light chain antisera, as well as a nonimmune rabbit serum. All were diluted in 1% bovine serum albumin. Slides were flooded with these reagents for 1 hour and washed in PBS. Bound antibody was visualized by exposure of the slides to a peroxidase substrate solution containing the chromagen 3,3′ diaminobenzidine (DAB). Lerner-3 hematoxylin (Lerner Labs, New Haven, CT) was used as the counterstaining reagent, and slides were mounted using a nonaqueous mounting medium (ACCU·Mount 60™, Scientific Products, McGaw Park, IL).

Formalin-fixed, paraffin-embedded bone marrow was cut on a microtome to a thickness of 6 μm and mounted on 0.01% poly-L lysine-coated slides. The sections were deparaffinized using a xylene-related solvent (AmeriClear™, Scientific Products) and brought through a series of graded alcohols to PBS. Sections were washed extensively and to block endogenous peroxidases, slides were submerged in 0.15% hydrogen peroxide/anhydrous methanol for 30 minutes. Next the bone marrow was pretreated with ficin (GammaZyme-F™, Gamma Biologicals, Inc., Houston, TX) for 30 minutes at 37° C. The sections were overlaid with appropriate dilutions of rabbit anti-human V_L antisera and incubated overnight at 4° C. Detection and visualization of bound antibody was accomplished using the ABC method, with DAB used as the substrate chromagen.

Clinical analyses were done using a direct and an indirect immunoperoxidase method. Cells or tissue from patients were routinely examined with commercially available primary reagents, which included peroxidase-conjugated sheep polyclonal anti-κ and anti-λ light chain antisera and anti-μ, -δ, -γ, and -α heavy chain antisera (Tago, Inc., Burlingame, CA) and with unconjugated mouse monoclonal anti-LEU 1 (CD5), -LEU 14 (CD22), and -LEU 4 (CD3) antisera (Becton-Dickinson, Mountain View, CA). For the monoclonal antibodies, a biotin-labeled, horse anti-mouse IgG antiserum (Vector) was used as the secondary reagent.

Proteins

An agarose gel immunofixation procedure (Paragon Electrophoresis System, Beckman, Brea, CA) was used routinely to examine serum and urine specimens for the presence of monoclonal Ig components. Because of the generally low amounts of urinary Bence Jones proteins in patients with CLL or malignant lymphoma (in contrast to those with multiple myeloma), 100-ml volumes of randomly collected or 24-hour urine specimens were dialyzed exhaustively against deionized, distilled water and then lyophilized. Before electrophoresis and immunofixation, the urine specimens were reconstituted in water to a final protein concentration of 50 mg/ml. The V_L subgroup of monoclonal urinary κ or λ chains was determined by comparative immunodiffusion analyses using anti-V_L subgroup-specific antisera.[4] For these analyses, urine specimens were appropriately diluted to yield a final monoclonal light chain concentration of 0.5 to 2 mg/ml.

Table 1. *Reactivity by ELISA of Polyclonal Anti-human V_κ and V_λ Subgroup-specific Antisera Used for Immunophenotyping Analyses**

Proteins	Anti-κ (R 360)†	Anti-$V_{\kappa I}$ (R 374)	Anti-$V_{\kappa II}$ (R 538)	Anti-$V_{\kappa III}$ (R 294)	Anti-$V_{\kappa IV}$ (R 178)	Proteins	Anti-λ (R 358)	Anti-$V_{\lambda I}$ (R 505)	Anti-$V_{\lambda II/V}$ (R 583)	Anti-$V_{\lambda III}$ (R 269)	Anti-$V_{\lambda IV}$ (R 510)	Anti-$V_{\lambda VI}$ (R 394)
κI	+	+	0	0	0	λI	+	+	0	0	0	0
κII	+	0	+	0	0	λII/V	+	0	+	0	0	0
κIII	+	0	0	+	0	λIII	+	0	0	+	0	0
κIV	+	0	0	0	+	λIV	+	0	0	0	+	0
						λVI	+	0	0	0	0	+

* Reactivity of proteins designated as + or 0 based on at least a >2.5-fold difference in optical density at 405 nm.
† Antiserum designation.

Patients

The diagnosis of CLL was made on the basis of established hematologic criteria and that of low-, mid-, or high-grade malignant lymphoma on the basis of morphologic criteria according to the Working Formulation. With rare exception, specimens for analyses were obtained before chemotherapy or radiotherapy was begun.

The B-cell nature of the cell populations obtained from 40 patients with CLL and other low-grade malignant lymphomas and 13 patients with mid- or high-grade lymphomas was evidenced by their $CD22^+$, $CD3^-$ reactivity and by the presence of sIgM or, less commonly, sIgM and sIgD. CD5 expression was limited to the CLL/low-grade populations, in which 37 of the 40 cases were found to be positive.

Results

Specificity of Anti-light Chain Antisera

The anti-light chain antisera used as primary antibodies for the immunocytochemical analyses of cellular Ig included two sets of reagents. One, designated 'anti-κ' or 'anti-λ,' recognized, respectively, antigenic determinants common to all κ or λ light chains. The second, designated 'anti-V_k' or 'anti-V_λ,' recognized V region determinants unique to each of the known human V_L subgroups, ie, anti-V_L subgroup-specific antisera.

The specificity of these anti-κ/anti-λ and anti-V_L subgroup antisera was unambiguously demonstrated by ELISA. For these analyses, the anti-light chain antisera were tested against a panel of monoclonal κ- and λ-type light chains as well as intact IgG, IgA, and IgM proteins of known V_L subgroups, as established by amino acid sequence.[1,4] As indicated in Table 1, the anti-κ antiserum recognized all κ-type Igs, regardless of V_κ subgroup, whereas the anti-$V_{\kappa I}$, anti-$V_{\kappa II}$, anti-$V_{\kappa III}$, and anti-$V_{\kappa IV}$ antisera were specific for κI, κII, κIII, and κIV type Igs, respectively. Similarly the anti-λ antiserum reacted with all λ-type Igs, whereas the anti-$V_{\lambda I}$, anti-$V_{\lambda II/V}$, anti-$V_{\lambda III}$, anti-$V_{\lambda IV}$, and anti-$V_{\lambda VI}$ antisera recognized only λI, λII/V, λIII, λIV, and λVI type Igs, respectively. Further confirmation of the specificity of these reagents resulted from immunoblotting experiments in which monoclonal Igs of known V_κ or V_λ subgroup were subjected to SDS polyacrylamide gel electrophoresis before subtyping analyses. Immunocytochemically the specificity of the anti-V_κ and anti-V_λ antisera also was apparent in blocking experiments in which cytospin preparations were overlaid before antisera application with monoclonal light chains representative of each V_L subgroup. The reactivity of a particular anti-V subgroup-specific antiserum was abrogated by subgroup-concordant monoclonal light chains but not by light chains of other subgroups.

Immunocytochemical Determination of V_L Subgroups

Multiple Myeloma, Amyloidosis AL, and Waldenström's Macroglobulinemia

Our initial efforts were directed toward the determination of the V_κ or V_λ subgroup of Ig molecules present in the cytoplasm of monoclonal plasma cell populations. For this purpose, we used plasma cell-enriched cytospin specimens isolated from the bone marrow or peripheral blood of patients with multiple myeloma who had serum or urinary monoclonal Igs. Sufficient numbers of slides were prepared to permit the application of normal (nonimmune) rabbit serum and, depending on the κ- or λ-type of the cytoplasmic Ig (as determined with the general anti-κ and anti-λ chain antisera), of the 4 anti-V_κ or 5 anti-V_λ subgroup-specific antisera. For comparative purposes and to eliminate nonspecific background staining, the slides were incubated with a minimum of three serial dilutions of each antiserum. Similar methods were used to examine, after deparaffinization and enzyme treatment, formalin-fixed, paraffin-embedded bone marrow specimens. As illustrated in Figures 1A and B, the V_L subgroup nature of the cIg was evident among the κ- and λ-type monoclonal plasma cells examined (no cases of the rare $V_{\lambda IV}$ sub-

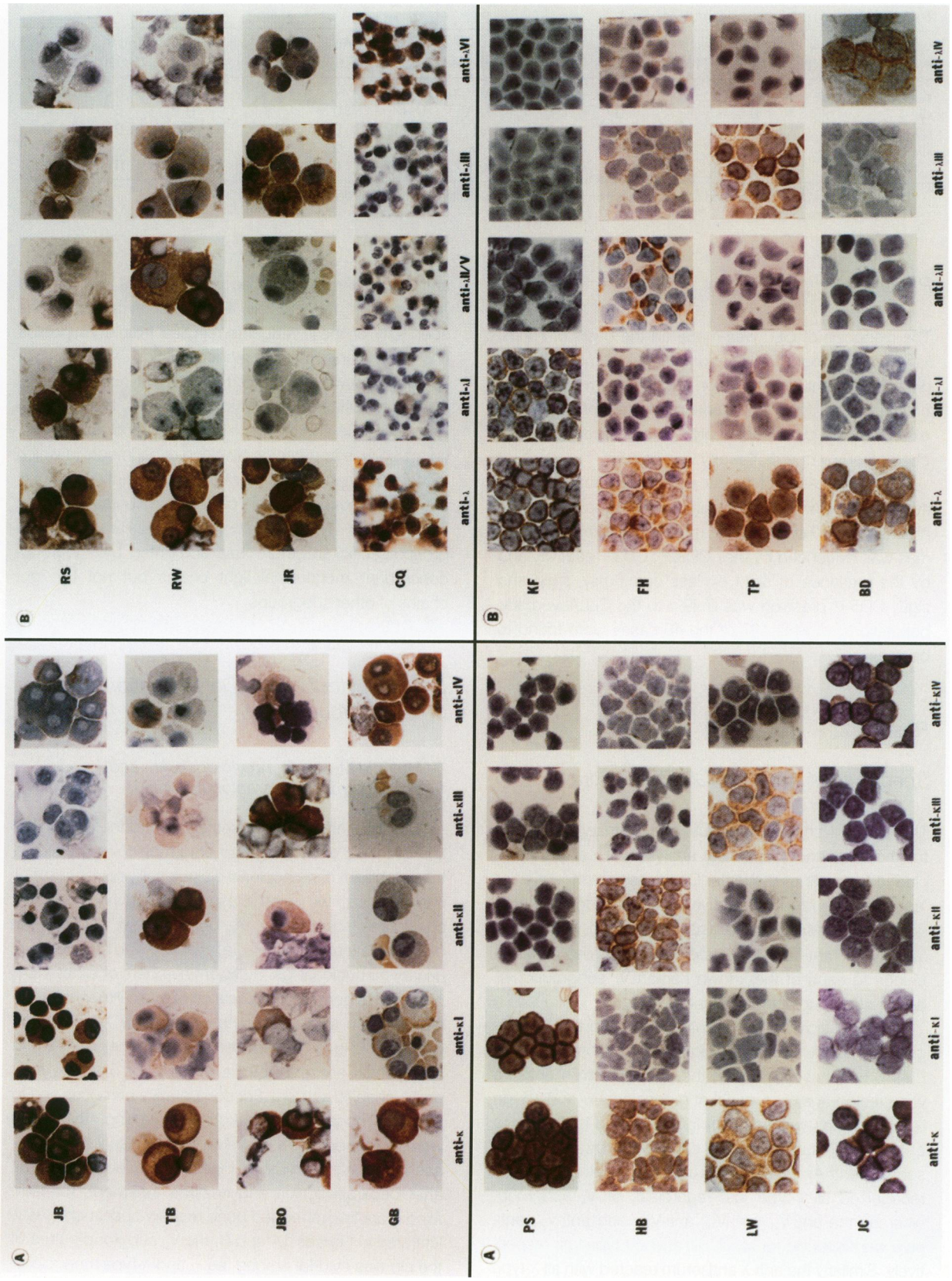
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Figure 1. *Immunocytochemical characterization of the V_κ and V_λ subgroups expressed by the light chains of cytoplasmic Ig (cIg) in monoclonal plasma cell populations. Bone marrow-derived plasma cells from patients with multiple myeloma (cytospin preparations) or amyloidosis AL (deparaffinized, enzyme-treated sections) examined using the ABC immunoperoxidase technique and the anti-light chain antisera as indicated; hematoxylin counterstain; ×1000.* **A (Top left):** *Kappa. Reactivity with anti-κ and anti-$V_{\kappa I}$, -$V_{\kappa II}$, -$V_{\kappa III}$, and -$V_{\kappa IV}$ antisera indicated the κI, κII, κIII, or κIV nature of the cIg in cells from multiple myeloma patients JB, TB, JBO, and GB, respectively.* **B (Top right):** *Lambda. Reactivity with anti-λ and anti-$V_{\lambda I}$, -$V_{\lambda II/V}$, -$V_{\lambda III}$, and -$V_{\lambda VI}$ antisera indicated the λI, λII/V, λIII, or λVI nature of the cIg in cells from multiple myeloma patients RS, RW, and JR and amyloidosis AL patient CQ, respectively (all patients' cells were tested with the anti-$V_{\lambda IV}$ antiserum and found to be negative).* Figure 2. *Immunocytochemical characterization of the V_κ and V_λ subgroup expressed by the light chains of surface Ig (sIg) in chronic lymphocytic leukemia. Cytospin preparations of peripheral blood-derived lymphocytes examined using the ABC immunoperoxidase technique and the anti-light chain antisera as indicated; hematoxylin counterstain; ×1000.* **A (Bottom left):** *Kappa. Reactivity with anti-κ and anti-$V_{\kappa I}$, -$V_{\kappa II}$, -$V_{\kappa III}$, and -$V_{\kappa IV}$ antisera indicated the κI, κII, κIII, or κIV nature of the sIg in cells from CLL patients PS, HB, LW, and JC, respectively.* **B (Bottom right):** *Lambda. Reactivity with anti-λ and anti-$V_{\lambda I}$, -$V_{\lambda II/V}$, -$V_{\lambda III}$, and -$V_{\lambda IV}$ antisera indicated the λI, λII/V, λIII, or λIV nature of the sIg in cells from CLL patients KF, FH, TP, and BD, respectively (all patients' cells were tested with the anti-$V_{\lambda VI}$ antiserum and found to be negative).*

group[5] were available in this population for study). In studies of cells obtained from 20 patients with multiple myeloma, plasma cell leukemia, or amyloidosis AL, there was complete concordance between the immunocytochemical V_κ or V_λ subgroup classification and that obtained through serologic classification of the patient's monoclonal serum Ig (IgG or IgA) or urinary Bence Jones protein.

We also examined bone marrow- and pleural fluid-derived lymphocytoid plasma cells obtained from four patients with Waldenström's macroglobulinemia. In such cases, it was also possible to identify the V_κ or V_λ subgroup nature of the cIgM. The cIg V_L subgroup determined immunocytochemically correlated with that found for the light chains of the secreted IgM protein.

Chronic Lymphocytic Leukemia and Other B-cell–Related Neoplasms

The immunocytochemical methodology used for determination of the V_L subgroup of cIg also proved suitable for analyses of sIg in other types of monoclonal B-cell populations. Lymphocyte-enriched (more than 90%) preparations were obtained by cytocentrifugation of heparinized peripheral blood or bone marrow specimens from patients with CLL and from cell suspensions prepared from the lymph nodes, tumor masses, or pleural fluid of patients with malignant lymphoma. Using the general anti-κ and anti-λ chain antisera, the virtually exclusive sIgκ$^+$ or sIgλ$^+$ nature of these cells was shown. Through the application of the anti-V_κ or anti-V_λ subgroup-specific antisera, it was possible to determine immunocytochemically the particular V_L subgroup expressed by these homogeneous populations of cells (as is illustrated for CLL cells in Figures 2A and B). In contrast, an admixture of sIgκ$^+$ and sIgλ$^+$ cells was found when samples from two patients with B-cell lymphoproliferative disorders were analyzed with the general anti-κ and anti-λ reagents. In each case the demonstration that the sIgκ or sIgλ cells expressed, respectively, a single V_κ or V_λ subgroup permitted the establishment of the monoclonal nature of the disease.

The anti-V_L subgroup-specific antisera also were used successfully to analyze intact sections of lymph node biopsies from patients with B-cell malignancies. In comparative studies involving five patients, the V_L phenotype was as apparent for cells present in fresh-frozen specimens as it was for the isolated cells in cytospin preparations.

Although we have found considerable variation in the intensity of sIg staining using the immunoperoxidase technique, we have been able to classify the V_L subgroup expressed by the sIg in 53 of 60 patients studied to date. For those patients having a detectable urinary monoclonal Ig, there was agreement between the V_κ or V_λ subgroup of the sIg and that of the excreted Ig. For the 40 patients with CLL and other low-grade lymphomas, 28 of these cases were sIgκ$^+$. Of these, 17 were classified as $V_{\kappa I}$, 4 as $V_{\kappa II}$, 4 as $V_{\kappa III}$, and 3 as $V_{\kappa IV}$. The 12 sIgλ$^+$ cases included 1 classified as $V_{\lambda I}$, 1 as $V_{\lambda II/V}$, 7 as $V_{\lambda III}$, and 3 as $V_{\lambda IV}$; no $V_{\lambda VI}$$^+$ cells were found among the sIgλ$^+$ patients. Among the 13 patients with mid- or high-grade lymphomas, 8 sIgκ$^+$ cases included 3 $V_{\kappa I}$, 1 $V_{\kappa II}$, 2 $V_{\kappa III}$, and 2 $V_{\kappa IV}$; for the 5 sIgλ$^+$ patients, 3 were classified as $V_{\lambda I}$, and 2 as $V_{\lambda III}$ (Table 2).

The percentage distribution of the V_κ and V_λ subgroups for all 53 patients (36 Igκ$^+$ and 17 Igλ$^+$) is given in Table 3 and is compared to the distribution of V_κ and V_λ subgroups found on serum Ig (based on analyses of more than 200 κ- and λ-type Bence Jones proteins[2,4]). Among the sIgκ$^+$ cases, the percentage of $V_{\kappa IV}$ was higher and that of $V_{\kappa III}$ was lower than expected. For the sIgλ$^+$ patients, a predominance of subgroups $V_{\lambda III}$ and $V_{\lambda IV}$, a decrease in $V_{\lambda II/V}$, and an absence of $V_{\lambda VI}$ were found.

Discussion

Heretofore, the V_L subgroup nature of the Ig expressed by monoclonal B-cell populations found in patients with multiple myeloma and other types of malignant lymphoproliferative disorders has been based primarily on analyses of the biologic products of these cells; namely, myeloma proteins, Waldenström macroglobulins, and Bence Jones proteins. The extent of this information has been

Table 2. *V_κ and V_λ Immunophenotypes in Malignant Lymphoma*

Pathologic Classification*	Number	V_κ subgroup				V_λ subgroup				
		κI	κII	κIII	κIV	λI	λII/V	λIII	λIV	λVI
Low grade										
slgκ	28	17	4	4	3					
slgλ	12					1	1	7	3	0
Mid/high grade										
slgκ	8	3	1	2	2					
slgλ	5					3	0	2	0	0
Total	53	20	5	6	5	4	1	9	3	0

* International Working Formulation.

limited, however, due to the unavailability of serum or urine specimens or, as in the case of 'nonsecretory' forms of B-cell–related neoplasms, a paucity of circulating or excreted monoclonal Igs. Using a sensitive immunoperoxidase method and our highly specific anti-light chain antisera, we have demonstrated the feasibility of determining immunocytochemically the V_L subgroup expressed by slg as well as by clg. The methodology used has been effective for analyses of cytospin preparations of bone marrow, peripheral blood, or lymph node-derived plasma cells and lymphocytes, as well as of fresh-frozen or formalin-fixed, paraffin-embedded tissue. We also found this technique useful for discriminating between monoclonal and polyclonal B-cell–related disorders.

For patients with multiple myeloma, amyloidosis AL, or Waldenström's macroglobulinemia, the V_L subgroup nature of clg expressed by monoclonal plasma cells or lymphoplasmacytoid cells was readily determined, even in those patients with amyloidosis who typically have minimal bone marrow plasmacytosis.[19] In all cases studied, there was complete concordance between the clg V_L subgroup and that of the serum or urinary monoclonal Ig. Other than the demonstrated association of the $V_{\lambda VI}$ subgroup with amyloidosis AL[5,14] and the predominance of the $V_{\lambda II/V}$ subgroup among λ-type Waldenström macroglobulins,[5] an association between V_L subgroups and particular clinicopathologic features of disease remains to be established.

The V_L subgroup of slg expressed by monoclonal B-cell populations in patients with CLL and other types of B-cell malignancies could also be determined immunocytochemically. The distribution of slg V_κ or slg V_λ subgroups in this patient population, however, differed from that found from analyses of the monoclonal serum and urinary Ig products of patients with multiple myeloma, amyloidosis AL, and Waldenström's macroglobulinemia.[2,4,5] Our data showed that among 40 patients with CLL and other low-grade lymphomas and 13 with mid- or high-grade disease, certain V_L subgroups predominated while others were decreased or absent. For slgκ$^+$ cells, the percentage of $V_{\kappa IV}$ was higher and that of $V_{\kappa III}$ was lower than expected based on the percentage distribution of these V_κ subgroups among monoclonal Igs. Even more striking was the unusual V_L subgroup distribution among slgλ$^+$ cells in which a predominance of the $V_{\lambda III}$ and (rare) $V_{\lambda IV}$ subgroups was found. We attribute the absence of $V_{\lambda VI}$ cells to the apparent exclusive association of this subgroup with amyloidosis AL.[5,14]

Our immunocytochemical data thus provide evidence for the predominance of certain V_L subgroups in B-cell neoplasms. One such subgroup, $V_{\kappa IV}$, is of interest because of the preferential association of κIV light chains with certain types of cold agglutinins, anti-intermediate filaments, and other types of autoantibodies.[2] The significance of the $V_{\lambda III}$ and $V_{\lambda IV}$ subgroup dominance among the slgλ$^+$ population studied is presently unknown, although it should be noted that a recently characterized monoclonal IgMλ rheumatoid factor also contained λIII-type light chains.[20] Additional studies of monoclonal slgκ$^+$ and slgλ$^+$ populations will be required to substantiate the apparent over-representation and clinical relevance of particular slg V_L subgroups in B-cell disorders.

Cells from virtually all our patients with CLL and low-grade lymphomas expressed, as expected,[21] the CD5

Table 3. *Comparison of the V_L Subgroup Distribution (%) Found on Serum Igκ and Igλ with that Found on the Cells of 36 Patients with slgκ$^+$ and 17 Patients with slgλ$^+$ B-cell–Related Malignancies*

Source	V_κ subgroup				Source	V_λ subgroup				
	κI	κII	κIII	κIV		λI	λII/V	λIII	λIV	λVI
Serum (Igκ) %*	56	10	30	4	Serum (Igλ) %*	26	38	22	3	11
Lymphoma (Igκ) %	55	14	17	14	Lymphoma (Igλ) %	23	6	53	18	0

* Based on serologic analyses of >200 κ and λ Bence Jones proteins.[2,4]

marker. This pan T-cell antigen is infrequently found on a relatively small population of normal tonsillar or spleen-derived B cells[22] but is commonly detected on low-grade neoplastic B-cell populations[23,24] as well as on auto-reactive lymphocytes.[25–28] Through studies of $CD5^+$, slgκ CLL cells and IgMκ autoantibodies, conservation in light (and heavy) chain V gene usage has been demonstrated for certain forms of malignant and reactive B-cell populations.[13,15–18,29,30] Remarkably the κ chains found on the slg of malignant B cells in ~25% of patients with $CD5^+$, slgκ CLL (and other low-grade, small cell lymphomas) and the light chains of IgMκ rheumatoid factors are members of the $V_{\kappa IIIb}$ sub-subgroup, express a cross-reactive idiotype (CRI) designated 17.109, and are products of the *Humkv* 325 germ-line gene.[11–13] Furthermore the resistance of this gene to somatic mutation has been evidenced by the virtual sequence identity of cDNA from cases of $CD5^+$, 17.109^+ B-cell malignancies and also by the strikingly homologous κIIIb protein sequences of *Humkv* 325-encoded 17.109^+ IgMκ autoantibodies.[15–18] In contrast, other morphologic types of B-cell lymphomas (eg, follicular cell) are usually $CD5^-$ and rarely express the 17.109 CRI; in such cases, restriction in Ig V gene usage and conservation has not been apparent.[17] Other examples of conserved V_L and V_H genes have been reported in patients with monoclonal B-cell neoplastic and autoimmune diseases.[31,32]

The demonstration that the V_L (or V_H) genes of monoclonal $CD5^+$ B cells are relatively resistant to somatic mutation (in contrast to light and heavy chain genes in malignant plasma cell and other cytomorphologic types of B-cell neoplasms) has given new insight into the pathogenesis of CLL and related lymphocytic malignancies. Our finding that the V_L subgroup distribution differs in multiple myeloma *versus* lymphocytic malignancies provides further evidence of fundamental biologic, as well as clinical, differences between these diseases. The ability to identify immunocytochemically the V_L nature of the Ig expressed by monoclonal B-cell populations offers a rapid and sensitive means to determine V_L gene usage in neoplastic and reactive disorders. The demonstration that particular V_L genes are preferentially associated with specific B-cell–related diseases has pathophysiologic importance, as well as diagnostic and therapeutic implications.

References

1. Kabat EH, Wu TT, Reid-Miller M, Perry HM, Gottesman KS: Sequences of Proteins of Immunological Interest, 4th Edition. U.S. Department of Health and Human Services (PHS, NIH), 1987, pp 41–77
2. Solomon A: Light chains of immunoglobulins: Structural-genetic correlates. Blood 1986, 68:603–610
3. Zachau HG: Immunoglobulin light-chain genes of the κ type in man and mouse. *In* Honjo T, Alt FW, Rabbitts TH, eds. Immunoglobulin Genes. London, Academic Press, 1989, pp 91–110
4. Solomon A: Light chains of human immunoglobulins. Methods Enzymol 1985, 116:101–121
5. Solomon A, Weiss DT: Serologically defined V region subgroups of human λ light chains. J Immunol 1987, 139:824–830
6. Solomon A, McLaughlin CL: Bence Jones proteins and light chains of immunoglobulins. IV. Immunochemical differentiation among proteins within each of the three established κ-chain classes. J Immunol 1971, 106:120–127
7. Roelcke D: Cold agglutination. Antibodies and antigens. Clin Immunol Immunopathol 1974, 12:266–280
8. Kunkel HG, Winchester RJ, Joslin FG, Capra JD: Similarities in the light chains of anti-γ-globulins showing cross-idiotypic specificities. J Exp Med 1974, 139:128–136
9. Ledford DK, Goñi F, Pizzolato M, Franklin EC, Solomon A, Frangione B: Preferential association of κIIIb light chains with monoclonal human IgMκ autoantibodies. J Immunol 1983, 131:1322–1325
10. Pons-Estel B, Goñi F, Solomon A, Frangione B: Sequence similarities among κIIIb chains of monoclonal human IgMκ autoantibodies. J Exp Med 1984, 160:893–904
11. Carson DA, Fong S: A common idiotope on human rheumatoid factors identified by a hybridoma antibody. Mol Immunol 1983, 20:1081–1087
12. Fong S, Chen PP, Gilbertson TA, Fox RI, Vaughan JH, Carson DA: Structural similarities in the κ light chains of human rheumatoid factor paraproteins and serum immunoglobulins bearing a cross-reactive idiotype. J Immunol 1985, 135: 1955–1960
13. Radoux V, Chen PP, Sorge JA, Carson DA. A conserved human germline Vκ gene directly encodes rheumatoid factor light chains. J Exp Med 1986, 164:2119–2124
14. Solomon A, Frangione B, Franklin EC: Bence Jones proteins and light chains of immunoglobulins. Preferential association of the $V_{\lambda VI}$ subgroup of human light chains with amyloidosis AL(λ). J Clin Invest 1982, 70:453–460
15. Kipps TJ, Fong S, Tomhave E, Chen PP, Goldfien RD, Carson DA: High-frequency expression of a conserved κ light-chain variable-region gene in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 1987, 84:2916–2920
16. Kipps TJ, Tomhave E, Chen PP, Carson DA: Autoantibody-associated κ light chain variable region gene expressed in chronic lymphocytic leukemia with little or no somatic mutation. Implications for etiology and immunotherapy. J Exp Med 1988, 167:840–852
17. Kipps TJ, Robbins BA, Kuster P, Carson DA: Autoantibody-associated cross-reactive idiotypes expressed at high frequency in chronic lymphocytic leukemia relative to B-cell lymphomas of follicular center cell origin. Blood 1988, 72: 422–428
18. Pratt LF, Rassenti L, Larrick J, Robbins B, Banks PM, Kipps TJ: Ig V region gene expression in small lymphocytic lym-

phoma with little or no somatic hypermutation. J Immunol 1989, 143:699–705

19. Solomon A: Current developments in immunoglobulins. *In* Silber R, Gordon AS, LoBue J, Muggia FM, eds. Contemporary Hematology/Oncology, New York, Plenum Press, 1981, pp 399–465
20. Natvig JB, Thompson K, Randen I, Steinitz M, Taussig M, Beale D, Barker P, Sletten K, Waalen K, Förre O: Partial amino acid sequence analysis and variable subgroup determination (VH and VL) of a monoclonal rheumatoid factor derived from a rheumatoid arthritis patient. Scand J Rheumatol Suppl 1988, 75:127–132
21. Kipps TJ: The CD5 B cell. Adv Immunol 1989, 47:117–185
22. Freedman AS, Freeman G, Whitman J, Segil J, Daley J, Nadler LM: Studies of in vitro activated CD5+ B cells. Blood 1989, 73:202–208
23. Royston I, Majda JA, Baird SM, Meserve BL, Griffiths JC: Human T cell antigens defined by monoclonal antibodies: The 65,000-dalton antigen of T cells (T65) is also found on chronic lymphocytic leukemia cells bearing surface immunoglobulin. J Immunol 1980, 125:725–731
24. Wang CY, Good RA, Ammirati P, Dymbort G, Evans RL: Identification of a p69,71 complex expressed on human T cells sharing determinants with B-type chronic lymphatic leukemic cells. J Exp Med 1980, 151:1539–1544
25. Preud'homme JL, Seligmann M: Anti-human immunoglobulin G activity of membrane-bound monoclonal immunoglobulin M in lymphoproliferative disorders. Proc Natl Acad Sci USA 1972, 69:2132–2135
26. Plater-Zyberk C, Maini RN, Lam K, Kennedy TD, Janossy G: A rheumatoid arthritis B cell subset expresses a phenotype similar to that in chronic lymphocytic leukemia. Arthritis Rheum 1985, 28:971–976
27. Hardy RR, Hayakawa K, Shimizu M, Yamasaki K, Kishimoto T: Rheumatoid factor secretion from human Leu-1$^+$ B cells. Science 1987, 236:81–83
28. Casali P, Burastero SE, Nakamura M, Inghirami G, Notkins AL: Human lymphocytes making rheumatoid factor and antibody to ssDNA belong to Leu-1$^+$ B-cell subset. Science 1987, 236:77–81
29. Kipps TJ, Tomhave E, Pratt LF, Duffy S, Chen PP, Carson DA: Developmentally restricted immunoglobulin heavy chain variable region gene expressed at high frequency in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 1989, 86: 5913–5917
30. Chen PP, Siminovitch KA, Olsen NJ, Erger RA, Carson DA: A highly informative probe for two polymorphic Vh gene regions that contain one or more autoantibody-associated Vh genes. J Clin Invest 1989, 84:706–710
31. Chen PP, Robbins DL, Jirik FR, Kipps TJ, Carson DA: Isolation and characterization of a light chain variable region gene for human rheumatoid factors. J Exp Med 1987, 166:1900–1905
32. Siminovitch KA, Misener V, Kwong PC, Song Q-L, Chen PP: A natural autoantibody is encoded by germline heavy and lambda light chain variable region genes without somatic mutation. J Clin Invest 1989, 84:1675–1678

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