FREQUENT BICLONALITY AND Ig GENE ALTERATIONS AMONG B CELL LYMPHOMAS THAT SHOW MULTIPLE HISTOLOGIC FORMS

By MARK H. SIEGELMAN, MICHAEL L. CLEARY, ROGER WARNKE and JEFFREY SKLAR

From the Laboratory of Experimental Oncology, Department of Pathology, Stanford University School of Medicine, Stanford, California 94305

B cell lymphomas are generally believed to result from the monoclonal proliferation of neoplastic B cells derived from a single transformed lymphocyte (1, 2). In a significant proportion of cases, two different histologic types of lymphoma are noted in the same patient, when separate biopsy specimens are obtained, either simultaneously or at different times (3, 4). Conversion between histologic categories of lymphoma usually involves changes, over time, from small cell size, often follicular morphology, to a large cell with a diffuse appearance. Such changes often coincide with the development of more aggressive disease, and with shortened survival. Conversion between these histologic types of B cell lymphoma are commonly interpreted as representing "dedifferentiation" of cells within the original malignant clone, and emergence of more rapidly proliferating lines of tumor cells (3–9).

Recently (10–12), we discovered a number of multiclonal B cell lymphomas arising in two different clinical situations. The first group of lymphomas occurred among iatrogenically immunosuppressed recipients of cardiac transplants (10). By analyzing rearrangements of Ig gene DNA in biopsy specimens from these patients, we detected 2–6 distinct clones of malignant B cells in each patient from whom more than one biopsy specimen was available (11). The second group of lymphomas arose among patients who were immunologically indistinguishable from normal individuals, and who suffered from apparently conventional B cell tumors (12). However, only a portion of the tumor cells within single biopsies from these patients reacted with antibodies directed against Ig idiotypes on the surface of the tumor cells. These antibodies were used to separate the reacting and nonreacting subpopulations of tumor cells within the biopsy tissue, and DNA extracted from each subpopulation was analyzed for Ig gene rearrangements. Results of this analysis confirmed the separate clonal origins of tumor cells that bound antiidiotype, and those cells that failed to bind antibody.

In view of the above findings, we decided to reexamine the widespread assumption that changes in, or multiple patterns of tumor histology reflect evolution within a single neoplastic clone, as opposed to separate clonal origins of the histologically distinct tumor tissues. We examined biopsy specimens from seven cases of B cell lymphoma in which two or three specimens, obtained at one time or over intervals of time, showed differences in histologic patterns between specimens. Our analyses of Ig genes in these tissues revealed that histologically distinct biopsy samples contained different clones in three cases, and that deletions of DNA had occurred in the heavy chain genes between histologically distinct biopsies in two other cases. Only two of the seven cases showed structural preservation of all monoclonal Ig gene rearrangements between different sites or over time, regardless of changes in tumor histology.

Materials and Methods

Cases of B cell lymphoma were analyzed retrospectively and at random, with the sole criteria of selection being (a) the availability of two or more biopsy specimens from each patient, and (b) differences in histology having been recorded upon microscopic examination of two of the tissue specimens. Review of the limited clinical data available revealed no major or consistent differences in therapy or unusual features in the clinical course of the patients. All tissues had been frozen immediately upon removal from the patient by immersion in an isopentane–dry ice bath, and stored frozen in plastic capsules at −70°C for up to seven years before our study. Table I summarizes the clinical and histological data on these cases.

DNA was extracted from 10–20 mg of frozen tissue by methods described elsewhere (13). Purified, high molecular weight DNA was digested with one of several restriction enzymes, and analyzed by the Southern blot hybridization procedure (14) using ^{32}P-radiolabeled DNA hybridization probes. Methods for these procedures and preparation of the probes have been described in detail before (13, 15, 16). The origins of the DNA probes within various human Ig genes are illustrated in Fig. 1. The probe specific for the μ switch region $(S_\mu)^1$ consisted of a 2 kilobase (kb) Sac I fragment. A DNA fragment probe specific for the enhancer sequence of the heavy chain Ig gene (E_H) was derived by subcloning an 800 basepair Bgl II/Hind III fragment from the heavy chain joining region (J_H) probe.

Ig heavy and light chains that were associated with tumor cells of the biopsy specimens were determined by immune phenotyping of frozen tissue sections, using an immunoperoxidase method and antibodies against κ, λ, μ, δ, γ, and α chains, as described previously (21, 22). Cellular Ig were not characterized beyond class and type (e.g., for idiotypes).

Results

Table I summarizes the data obtained from analyses of biopsy tissues. In five of the cases studied (cases 2–6), the histologic differences between tissue specimens involve changes in cell size (small vs. large cell lymphoma). Biopsy specimens from the two remaining cases (cases 1 and 7) differed in architectural features of microscopic anatomy (diffuse vs. follicular lymphoma). Six of the cases exhibited cellular Ig. These Ig were of the same heavy chain class and light chain type in each biopsy within the case, except for case 5, in which tissue of one biopsy contained λ light chain Ig, and a second biopsy specimen contained κ light chain Ig. Both biopsy specimens from case 3 lacked Ig detectable by immune phenotyping of frozen tissue sections.

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[1] *Abbreviations used in this paper:*   C, constant region; D, diversity-generating region; E_H, enhancer sequence of the heavy chain Ig gene; J_H, joining region of the heavy chain Ig gene; S, switch region; V, variable region.

TABLE I

Summary of Tissue Specimens from Patients Showing Multiple Histologic Categories of
Non-Hodgkin's Lymphoma

Case		Date	Biopsy site	Histologic diagnosis	Immunophenotype
1	(a)	10/31/80	Right axillary	Follicular large cell ML	μ, λ
	(b)	2/1/82	Para-aortic LN	Diffuse large cell ML	μ, λ
	(c)	2/1/82	Liver	Diffuse large cell ML	μ, λ
2	(a)	1/10/81	Supraclavicular LN	Small lymphocytic ML	μ, κ
	(b)	7/19/82	Right supraclavicular LN	Diffuse large cell ML	μ, κ
3	(a)	12/9/81	Right preauricular LN	Diffuse small cleaved cell ML	Ig$^-$
	(b)	9/10/82	Presternal LN	Diffuse large cell immunoblas-tic ML	Ig$^-$
4	(a)	11/29/82	Abdominal LN	Diffuse large cell immunoblas-tic ML	μ, κ
	(b)	11/29/82	Spleen	Small lymphocytic ML	μ, κ
5	(a)	8/30/78	Left tonsil	Diffuse large cell ML	μ, λ
	(b)	9/7/78	Right posterior cervical LN	Follicular and diffuse small cleaved cell ML	μ, κ
	(c)	9/22/78	Para-aortic LN	Follicular and diffuse small cleaved cell ML	ND
6	(a)	9/25/79	Right axillary LN	Small lymphocytic ML	μ, λ
	(b)	9/8/80	Spleen	Small lymphocytic ML	μ, λ
	(c)	2/23/83	Left inguinal LN	Diffuse large cell ML	μ, λ
7	(a)	1/8/77	Para-aortic LN	Small lymphocytic ML	μ, κ
	(b)	12/18/81	Left supraclavicular LN	Follicular small cleaved cell ML	μ, κ

ML, malignant lymphoma; LN, lymph node; Ig$^-$, no cellular immunoglobulin detected; ND, not done.

Southern blot hybridizations using the J_H probe and the two light chain probes revealed clonal rearrangements of the heavy chain gene and at least one light chain gene in each biopsy (15). These results confirmed the diagnosis of B cell lymphoma in each biopsy. For all specimens, we found agreement between results of immune phenotyping and analysis of Ig gene rearrangements, such that a rearrangement was found in the corresponding light chain gene whenever expression restricted to that light chain type was found by immune phenotyping for cellular Ig.

In two of the seven cases examined (cases 1 and 2), the positions of bands that signified clonally rearranged genes were the same in multiple biopsy specimens from the patient for each of the respective heavy and light chain genes (data not shown). In addition, no more than two rearranged bands were found per gene in any of the specimens analyzed. This is the maximum number of rearranged bands possible for a given chain within a single clone of B cells if both alleles
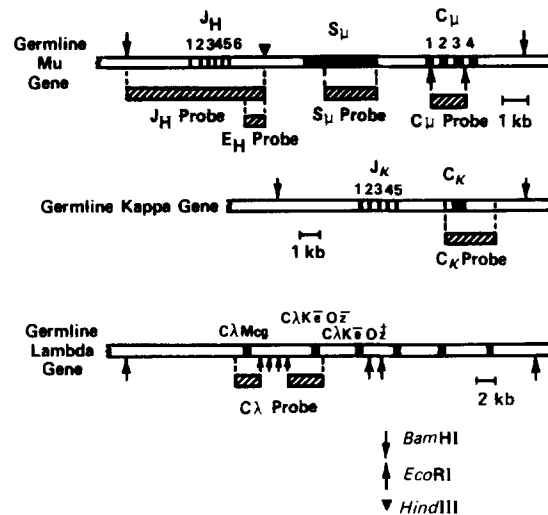
FIGURE 1. Ig gene DNA fragments used to analyze the configuration of cellular Ig gene DNA. Chromosomal DNA surrounding the constant regions and the relevant functional sites for each of three Ig genes are illustrated (17–20). DNA fragments used as hybridization probes in this work are indicated by crosshatched boxes below the chromosomal DNA of each Ig gene. The J_H probe consisted of a 6.5 kb Bam HI/Hind III DNA fragment that contains the entire J_H region, in addition to several kilobases of flanking DNA. The E_H probe consisted of an 800 basepair Bgl II/Hind III DNA fragment derived from the J_H probe. The S_μ probe consisted of a 2.0 kb Sac I DNA fragment. The C_μ probe was a 1.5 kb Eco RI fragment that contains a portion of the first, and all of the second and third exons of the C_μ coding sequence. The C_κ probe contained a 2.5 kb Eco RI fragment spanning the entire human C_κ. The C_λ locus consists of a family of at least six closely linked, related gene segments, encoding different allotypes (e.g., Mcg, Ke⁻Oz⁻, and Ke⁻Oz⁺). The combined C_λ probe was made up of a 3.5 kb Eco RI/Hind III fragment containing the Ke⁻Oz⁻ C_λ segment and a 2.5 kb Eco RI/Hind III fragment containing the Mcg C_λ segment. This probe detects all six nonallelic C_λ segments, and also weakly hybridizes with several putative C_λ pseudogenes.

have rearranged. The findings in this group of patients indicate, therefore that in each case a single monoclonal proliferation of B lymphocytes composed the tumor populations in both biopsies, despite disparity in histologic type, differences in anatomic sites of biopsy, and, in some cases, the passage of time between sampling of the tissues.

In three additional cases (cases 3–5), monoclonal patterns of rearranged, nongermline bands were again detected in Southern blot autoradiograms produced from each biopsy specimen (Fig. 2). However, in these cases, the positions of the bands in analyses for each gene varied one from the other. In other words, although only one clone appeared to constitute each tumor that was biopsied, the clones within these tumors were not the same.

In the last two cases (cases 6 and 7), analyses for light chain gene rearrangements disclosed rearranged, nongermline bands in identical positions for each of the multiple biopsies within each case (Fig. 3A and 4A). This result implied that these two cases represented monoclonal B cell lymphomas. In contrast to the patterns of bands obtained in analyses of light chain genes, the pattern of rearranged heavy chain bands that hybridized with the J_H probe differed between the biopsies within each case. To further characterize differences in the structure

of the heavy chain genes in cases 6 and 7, DNA was hybridized with probe S_μ, the constant μ region (C_μ), and E_H.

In analyses of DNA from all three biopsy specimens of case 6, both the S_μ and C_μ probes detected two rearranged, nongermline bands, one of which was not revealed by hybridization with the J_H probe. This finding was probably due to
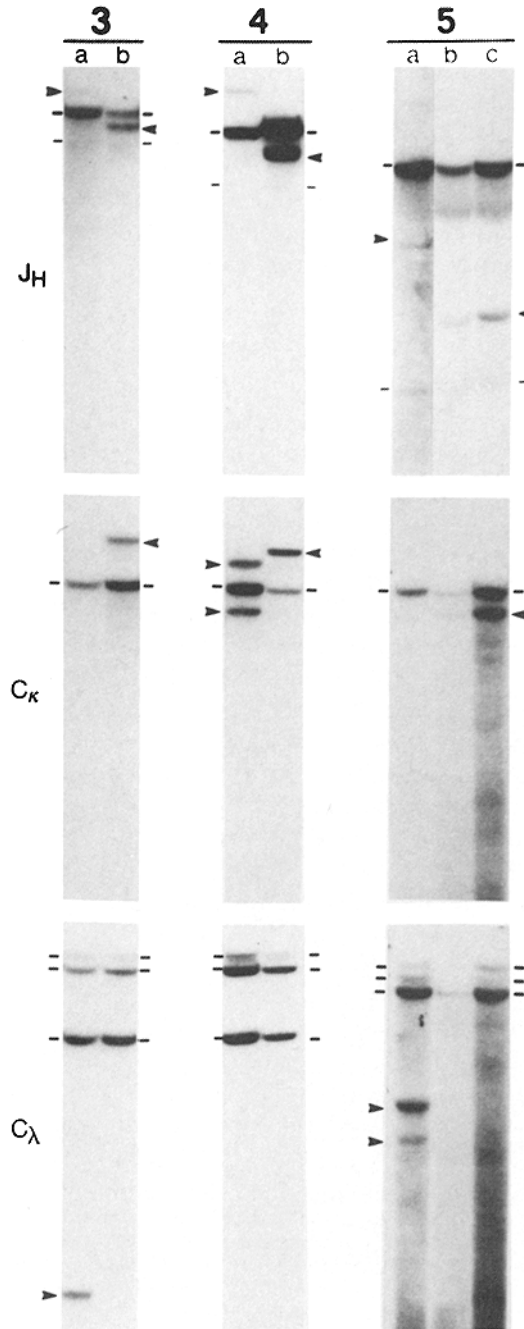
rearrangement of both heavy chain alleles in the cells of this lymphoma; however, in one allele, a defective, nonproductive rearrangement had probably occurred, resulting in deletion of all DNA sequences complementary to the J_H probe. The germline band in these autoradiograms was most likely contributed by contaminating nonlymphoid and/or nonlymphoma cells within the biopsies. In addition, one of the rearranged bands detected with the S_μ probe in the first two biopsies (a and b) was not detected in the third biopsy (c). Instead, a weakly hybridizing band corresponding to a DNA fragment slightly smaller than the unrearranged germline fragment, and containing at least some sequences homologous to the S_μ probe, was detected in DNA extracted from the third biopsy. Hybridization of the DNA from the third biopsy with the C_μ probe revealed a nongermline band that was in a position intermediate between the two rearranged bands of a and b, and that was not present in analyses from the two earlier biopsies. As noted above, analyses of light chain gene DNA revealed identical patterns of bands in all three biopsy specimens from case 6.

Taken together, these data suggest that in case 6 the three tumors were derived from the same clone of neoplastic B cells. However, at least a partial deletion of the S_μ DNA of the expressed heavy chain allele had occurred within all of the tumor cells present in the third biopsy (Fig. 3B). This interpretation is consistent with the hypothesis that the upper rearranged band, detected in the first two biopsies with the C_μ probe, "shifted" to a lower position in the autoradiogram obtained from the third biopsy specimen. Moreover, when a Hind III restriction digest was hybridized with the E_H probe, the same rearranged band was detected in all three biopsies, indicating that, in cells of different tissue samples, changes in the DNA of the corresponding allele must have taken place 3' to the Hind III site in this allele. The weakly hybridizing rearranged band that was detected with the S_μ probe in the third biopsy may reflect retention of a portion of the deleted S_μ DNA elsewhere in the genome. However, the actual origin of this band is unknown.

Three rearranged, nongermline bands were detected in hybridizations of the J_H probe with DNA extracted from both biopsy specimens of case 7 (Fig. 4A). The two largest rearranged bands in both biopsies occupied identical positions, while the third, and lowest bands differed from one another in their positions within the autoradiogram. When a similar restriction digest of DNA extracted from the first biopsy specimen was hybridized with the C_μ probe, the two larger rearranged bands were detected, but the smallest band could not be seen. The

FIGURE 2. Autoradiograms produced from DNA of biclonal lymphomas. Numbers and letters correspond to those in Table I. Analyses with the J_H and C_κ probes were performed on DNA digested with Bam HI restriction enzyme, except for analyses of case 5 with the J_H probe, which were performed on DNA digested with Hind III. Analyses with the C_λ probe were performed on DNA digested with Eco RI. Dashes indicate unrearranged germline bands, including polymorphic variant germline bands (23) in analyses of λ DNA for case 5. Approximate sizes of fragments corresponding to the germline bands, as determined from marker DNA fragments electrophoresed in adjacent lanes of the gel, are: 19 kb (major band) and 12 kb (crosshybridizing minor band) in J_H autoradiograms of cases 3 and 4; 10.5 kb (major band) and 3.5 kb (minor band) in the J_H autoradiogram of case 5; 12 kb in C_κ autoradiograms; and 16, 14, and 8 kb in C_λ autoradiograms, with a 19 kb polymorphic band in case 5. Arrows indicate clonally rearranged bands. The intensity of rearranged bands relative to germline bands varies with the amount of lymphoma present in a given tissue specimen.
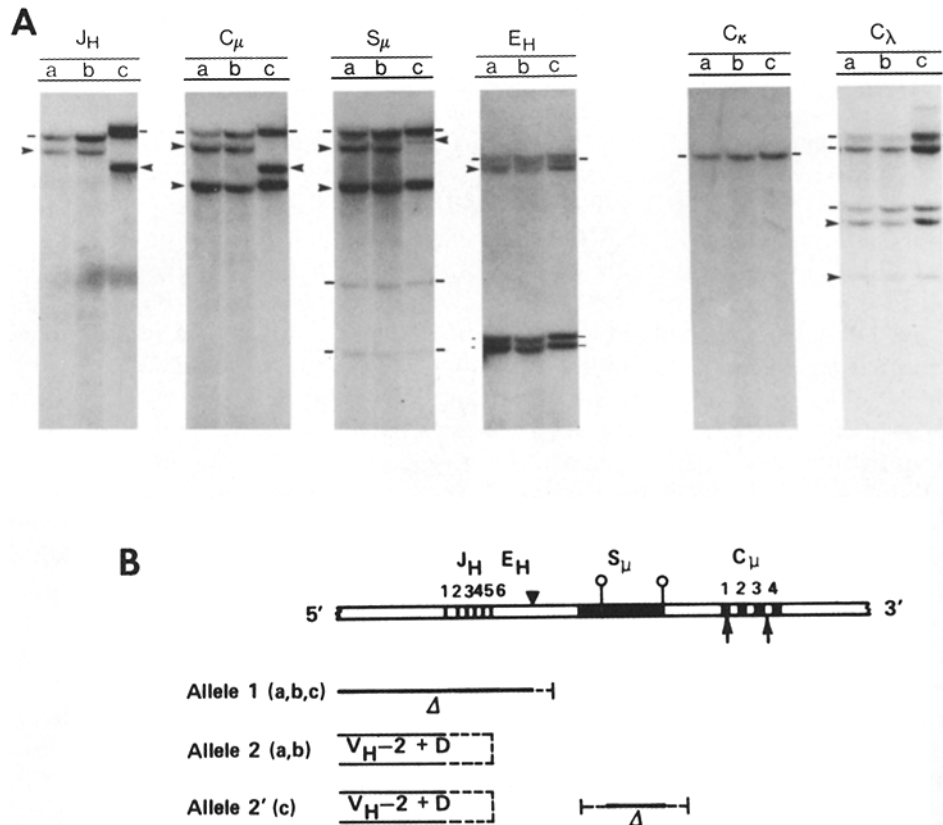
FIGURE 3. (A) Autoradiograms produced from DNA of case 6. Analyses with the J_H, C_μ, S_μ, and C_κ probe were performed on DNA digested with Bam HI restriction enzyme. Analysis with the E_H probe was performed on DNA digested with Hind III, and analysis of DNA with the C_λ probe was performed on DNA digested with Eco RI. Dashes and arrows have the same meaning as in fig. 2. The germline bands in the S_μ autoradiogram correspond to fragments of about 19, 4.4, and 2.5 kb; a 10.2 kb germline fragment is obscured by the lowest rearranged S_μ band (see fig. 4). The germline bands in the E_H autoradiogram correspond to fragments of]0.5, 3.5, and 3.3 kb in size. The precise origins of all germline bands in autoradiograms resulting from hybridizations with the S_μ and E_H probes are not known. Bands were labeled as germline based on their positions relative to markers that comigrated in the gels, and to the positions of bands obtained with nonlymphoid DNA from several patients. The major significance of results using these two probes is in the differences or similarities in patterns of bands between DNA of the two biopsy specimens in the case. (B) Map of rearrangements and deletions in the heavy chain loci of case 6 based on results in A. Open triangles indicate deletions of DNA. Open circles on stems indicate positions of Sac I restriction sites in the S_μ region; upgoing arrows and arrowheads indicate Eco RI and Hind III sites, respectively. V_H–2 and D indicate DNA of arbitrary V and D regions that have inserted into DNA near the C_μ region as a result of heavy chain gene rearrangement. Dashed portions of the figure indicate uncertain boundaries of deletions or sites of recombination.

latter band was nevertheless visualized when the same DNA digest was hybridized with the S_μ probe. Analyses of DNA obtained from the second biopsy specimen revealed the converse situation. The smallest rearranged band was seen in a DNA digest hybridized with the C_μ probe, but this band did not appear when DNA was hybridized with the S_μ probe. Analysis of light chain gene DNA showed no differences between the two clones.

Although a number of interpretations are possible for case 7, the simplest
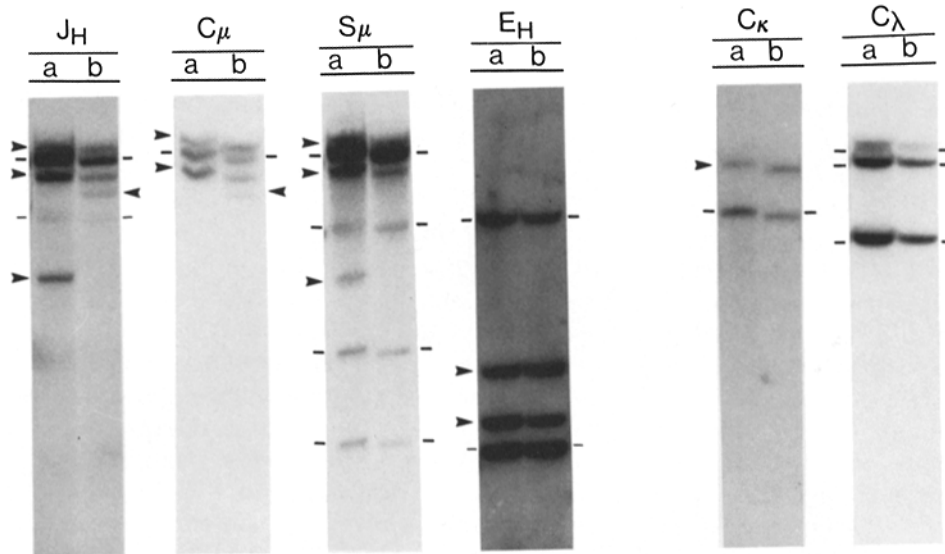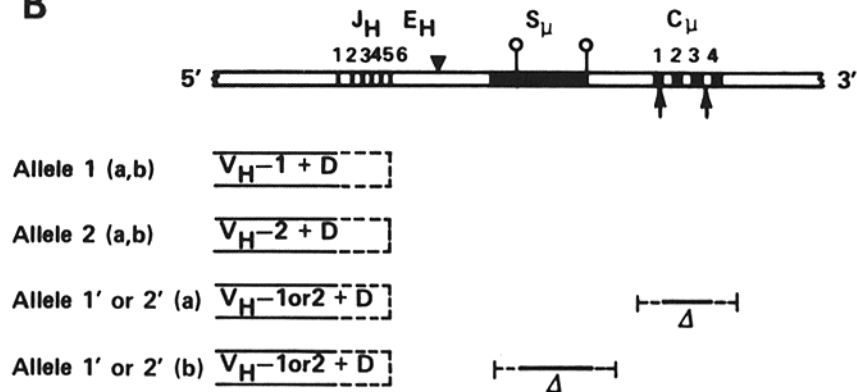
FIGURE 4.   (A) Autoradiograms produced from DNA of case 7. Analyses were carried out as in fig. 3. (B) Map of rearrangements and deletions in the heavy chain loci of case 7 based on the results in A. The symbols in both A and B have the same meaning as in fig. 3. The 3' endpoint of the C_μ deletion may extend far to the right.

explanation with which these results are compatible is that only one clone of neoplastic B cell was present in both biopsies from case 7. Both heavy chain alleles had rearranged within the cells of this clone. However, one of the heavy chain alleles in a subpopulation of the tumor cells of the first biopsy had also undergone a deletion of its C_μ region. In the second biopsy specimen, one of the heavy chain alleles in a subpopulation of the tumor cells had retained its C_μ region, but had deleted its S_μ region (Fig. 4B). Which of the two alleles contained the C_μ deletion and which allele contained the S_μ deletion is not clear from the

available data. Support for the conclusion that all nongermline bands arose from two basic rearrangements of variable (V), diversity (D), and J_H DNA comes from Hind III restriction digest of DNA hybridized with the E_H probe. This analysis detected two identical rearranged bands in both biopsies, demonstrating that both biopsy specimens in this case contain the same two configurations of DNA in the vicinity of J_H. Therefore, the third nongermline band in these biopsies most likely arose from postrearrangement deletions 3' to J_H, that is, in the S_μ and C_μ regions.

Discussion

The cases analyzed in this study can be separated into three different sets. In the first set were two cases in which multiple biopsies contained the same clones of proliferating B cell lymphoma, as assessed by Ig gene rearrangements, despite discrepancies in histology between the biopsy specimens. These cases conform to the conventional belief that changes in histology in B cell lymphoma between anatomic sites or over intervals of time represent morphologic changes in a single clone of tumor cells. Also, these findings demonstrate that rearranged configurations of Ig genes are often stable markers for malignant B cell clones. That configurations of Ig genes are frequently stable in certain tumors over long periods of time agrees with previous observations on the persistence of the same configuration of rearranged Ig genes in several actively dividing human B lymphoid cell lines maintained in culture for many months (11, 12, and our unpublished observations).

In the second set of cases, a change in histology between small cell and large cell type correlated with differences in clonal Ig gene rearrangements. No common rearrangements in any Ig gene were detected among biopsies from any of the three cases within this group. The neoplastic lymphocytes making up the tumor in the separate biopsy specimens therefore showed no relatedness on the basis of Ig gene rearrangements. By this criterion, the lymphomas, in these cases, were biclonal. Had additional biopsy material been available from these patients, possibly more neoplastic clones may also have been detected. These results contradict the belief that B cell lymphoma is always monoclonal, even when more than one histologic type is observed in one patient. Although the sample of cases analyzed in this study is relatively small, the incidence of two neoplastic B cell clones observed among these cases (43%) indicates that biclonality is not rare in lymphoma.

Of course, none of our data eliminates the possibility of a single malignant or premalignant progenitor cell, one which had not yet fully rearranged any of its Ig genes, for each of the apparently biclonal cases in our study, as we have discussed previously in other contexts (11, 12). A possible precedent for this type of phenomenon exists among tumors derived from lymphoid precursor cells isolated from fetal mouse livers and transformed in vitro by Abelson murine leukemia virus (A-MuLV). Some cells transformed in this manner have apparently undergone $D-J_H$ joining of their heavy chain alleles, and go on to rearrange randomly V_H and $D-J_H$ regions in culture (24, 25). Few of the cells in these cultures, however, proceed to rearrangement of light chain Ig genes.

The last group of cases in this study consisted of two patients whose lymphomas

showed structural changes in heavy chain alleles between successive biopsies. The majority of Ig genes in these cases, including all of the light chain alleles and perhaps one heavy chain allele in each case, showed retention of the same DNA configuration over time and between sites. Conservation of the same light chain rearrangements, and the presence of identical configurations of DNA surrounding the J_H region of the heavy chain gene in each biopsy suggest that the lymphomas in these two patients were monoclonal, as were those in the first set of cases.

In contrast to the maintained structure of the light chain alleles, various deletions in heavy chain alleles were detected between biopsies in both cases of this last set. In case 6, an apparent deletion of the S_μ region had occurred in the tumor cells of the last biopsy relative to those in the two earlier biopsies. In case 7, two possible heavy chain deletions were apparent; a deletion of the S_μ in one allele, and the deletion of the C_μ region in the same allele or possibly the other allele.

The structure of deletions at a detailed molecular level in these two cases cannot be deduced without cloning of the mutant alleles and nucleotide sequence analysis. In the meantime, a reasonable speculation is that repetitive nucleotide sequences within the S_μ DNA may account for a high rate of deletion of this region by homologous recombination. Also, deletion of the C_μ region by a class-switching event within the heavy chain locus cannot be excluded as an explanation for the second gene alteration seen in case 7. However, if class switching had occurred, the consequences of this process were not detected by immune phenotyping, which showed μ-κ Ig associated with the vast majority of tumor cells in biopsy specimens from case 7, and no significant amounts of other heavy chains among cellular Ig. Several other preliminary results also argue against deletion of C_μ DNA by class switching in case 7. For example, hybridization of DNA from this case with a DNA probe for the $S_{\gamma 4}$ region failed to reveal any nongermline bands (data not shown). Since this probe crosshybridizes with all S_γ and S_ϵ regions (I. Kirsh, National Cancer Institute, National Institutes of Health, Bethesda, MD, personal communication), these results suggest that heavy chain switching involving γ or ϵ loci is unlikely. However, switching to heavy chain classes other than γ and ϵ cannot be ruled out by these analyses. In addition, a Bam HI digest of DNA from specimen a of case 7 was hybridized with a probe that consisted of the 500 basepair Sac I fragment derived from DNA adjacent to the DNA of the S_μ probe and 3' to the S_μ region. This probe detected the identical pattern of rearranged bands that were found with the S_μ probe, and which are shown in Fig. 4A. Hypothetically, DNA homologous to the 500 basepair Sac I probe would have been deleted by a recombinational class-switching event. This deletion would have resulted in the absence from the autoradiogram obtained with the Sac I probe of the lowest rearranged band detected with the S_μ probe.

Possible internal deletions within heavy chain Ig genes have been encountered before in several different settings. For instance, monoclonal antibodies against heavy chains have been used to detect loss of heavy chain constant region domains from murine monoclonal antibodies after mutagenesis of a hybridoma that was propagated in vitro (26, 27). Also, early work on experimentally induced mouse myelomas demonstrated deletions of tryptic peptides from the Fc region of α

heavy chains produced by several subclones of the same primary plasmacytoma (28, 29). Whether or not loss of amino acids from the heavy chain proteins in any of these studies resulted from deletions in constant region DNA was not directly shown, so the degree of similarity to the C_μ deletions detected in case 7 remains to be determined. Another possible parallel to case 7 occurs among the paraproteins of human heavy chain disease. Evidence suggests (30) that at least some of the monoclonal heavy chains found in the sera of patients with this disease are missing constant region domains due to deletions of genomic DNA.

Deletion of the S_μ region DNA in two of our cases raises questions about the possible normal functional significance of this genetic alteration. For instance, could deletion of S_μ DNA serve to block heavy chain class switching after antigenic stimulation, and thereby ensure that some lymphocytes will be restricted to expression of IgM? Further studies will be necessary to investigate this possibility.

Chromosomal translocations involving breakpoints within the Ig heavy chain genes have been described recently (31, 32) in the cells of various types of B cell lymphoma. For instance, a consistent 14;18 translocation has been found in a majority of follicular lymphomas, and a 11;14 translocation has been noted (33, 34) in several cases of B cell lymphoma that showed conversion between small and large cell histologic types. Breakpoints in DNA around this locus may account for any of the nongermline heavy chain bands detected in Southern blot autoradiograms, including those bands believed to have arisen from deletions of the S_μ and C_μ regions. However, the possibility of translocations within lymphomas does not change the validity of our interpretations concerning the clonality of any cases in our study. If translocations are present in our cases and explain the observed deletions, they presumably produced loss of DNA sequence within the heavy chain gene, as well as interchromosomal recombination of DNA. Such translocations would also, necessarily, represent secondary events within a lymphoma, since they are not present either in the earliest biopsies of case 6, nor in all of the neoplastic cells within either biopsy specimen of case 7. Unfortunately, the retrospective nature of this study precluded karyotype analysis of the lymphoma tissues. However, when DNA probes become available for these specific chromosomal translocations, testing for translocations in these tumors will be possible by Southern blot analysis.

Recently, a report appeared that described a single case of Richter's syndrome (development of lymphoma in a patient previously diagnosed with chronic lymphocytic leukemia) in which the lymphoma cells expressed different Ig light chains, and possessed heavy chain gene rearrangements different from that of the leukemic cells (35). Because of these findings, and the 23 year interval between diagnosis of the leukemia and detection of the lymphoma, the authors of this report concluded that their case represented the development of independent and unrelated lymphoid neoplasms in a single patient. A similar interpretation is equally possible for our three cases of biclonal lymphomas. However, the high frequency with which biclonal lymphomas arise suggests that the occurrence of two clonally distinct neoplasms is more than coincidence. A biological link between the two clones seems likely, and a common malignant or premalignant progenitor for both clones may be such a link.

Our unexpected finding of deletions in rearranged heavy chain alleles raises a cautionary note regarding diagnosis of biclonal lymphoma based on presumed Ig gene rearrangements. That deletions can apparently occur in previously rearranged heavy chain genes indicates that the configuration of DNA within an Ig gene is not always fixed once rearrangement of constant and variable regions has taken place. As a result, deletions may give rise, in Southern blot autoradiograms, to nongermline bands that superficially appear to be due to unrelated, de novo gene rearrangement, rather than to mutations in pre-existing rearrangements. Therefore, if only heavy chain genes are compared between lymphoma biopsies, monoclonal disease with deletions in a previously rearranged allele may be mistaken for biclonal lymphoma. On the other hand, no data presently exists for postrearrangement light chain gene alterations in human lymphoma DNA. Consequently, apparent differences in heavy chain gene rearrangements between biopsy specimens, in the absence of differences in light chain gene rearrangements, should be regarded as insufficient evidence for biclonality, without further investigation.

Summary

Configurations of Ig gene DNA were examined in multiple biopsy specimens from seven cases of human B cell lymphoma that showed histologic differences among the specimens within each case. Analysis by Southern blot hybridizations with DNA probes for each of the three Ig loci revealed that the configurations of DNA within these loci were identical among the specimens in two of the cases. This result indicated the monoclonality of these lymphomas, despite differences in histology between biopsy specimens. In contrast, no common nongermline configurations of Ig gene DNA were detected among multiple biopsies in each of three other cases. Therefore, different histologies correlated with separate clones of proliferating B cells in these cases. In the last two cases, the configurations of light chain gene DNA were the same among biopsies in each case, consistent with a monoclonal origin in both lymphomas. However, differences were detected in the configuration of the heavy chain gene DNA. Analysis with a series of DNA probes of the μ heavy chain region indicated that the differences in the DNA configurations of the heavy chain genes from the biopsies probably arose from postrearrangement deletions of either the switch or constant regions of the μ gene. These studies indicate that, contrary to the conventional belief, individual tumors that contain different histologic types of lymphoma within the same patient frequently arise from separate clones of neoplastic cells. Furthermore, the heavy chain genes of monoclonal tumors may show postrearrangement deletions, often resulting from instability of DNA sequences within or around the μ switch region.

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