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RAPID COMMUNICATION

Rearrangement of κ-Chain and T-Cell Receptor β-Chain Genes in Malignant Lymphomas of "T-Cell" Phenotype

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Detection of immunoglobulin gene rearrangements by molecular hybridization is considered to be a highly sensitive approach to the evaluation of clonality of B-cell–derived neoplasms. Like monoclonal surface immunoglobulin in B cells, which serves as a reliable immunophenotypic marker for clonality, rearrangement of the genes encoding immunoglobulin light chains has been accepted as a reliable genotypic marker for the presence of a clonal expansion of B lymphocytes. The authors now report 3 cases of non-Hodgkin's lymphoma that were immunologically classified as having a T-cell phenotype and in which, in addition to rearrangements of the T-cell receptor β-chain gene, a rearrangement of an immunoglobulin light-chain gene was clearly identified by Southern blot hybridization. The results demonstrate that the data obtained by molecular hybridization should be interpreted with caution when the immunogenetic findings do not correlate with immunophenotypic expression, and that the results of molecular genetics studies should be interpreted in conjunction with morphologic and immunologic findings. (Am J Pathol 1987, 129:201–207)

RECENTLY, the value of immunogenetic analyses for the diagnosis and characterization of human malignant lymphomas has been established. In particular, rearrangement of the T-cell receptor (TCR) genes and of immunoglobulin heavy- and light-chain genes have been demonstrated to be highly sensitive and reproducible markers for the determination of clonality and/or lineage of non-Hodgkin's lymphomas (NHL).[1–10] In the course of evaluating a series of NHLs for rearrangements of the immunoglobulin heavy- and light-chain genes, and of the TCR β-chain (TCRβ) genes, we identified 3 cases of lymphoma that were characterized as T-cell by immunologic methods but were found to have unexpected and unusual immunogenetic features.

Materials and Methods

Patients and Samples

The 3 cases of diffuse NHL which form the basis of this study were received in consultation at the James Irvine Center for the Study of Leukemia and Lymphoma at the City of Hope National Medical Center. The laboratory research protocol was approved by the Institutional Review Board of the City of Hope.

Case 1

The patient was a 5-year-old boy who had a mediastinal mass and airway obstruction. A diagnosis of

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lymphoblastic lymphoma was established after a lymph node biopsy. There was no peripheral blood or bone marrow involvement at the time of diagnosis. The patient received combination chemotherapy and achieved a complete remission.

Case 2

The patient was a 67-year-old man who had generalized lymphadenopathy, hepatosplenomegaly, hemolytic anemia, and a strongly positive reaction in the direct Coombs test. A lymph node biopsy was performed, and a diagnosis of diffuse mixed cell lymphoma was made.

Case 3

A 47-year-old man had an enlarged inguinal lymph node. A computerized tomographic scan revealed a retroperitoneal mass. A biopsy was performed on the inguinal lymph node, and a diagnosis of diffuse large cell lymphoma was made. The patient received combination chemotherapy and radiation to the pelvis, which resulted in a complete remission.

Tissue Processing and Immunohistologic Techniques

The preparation of tissue samples for morphologic and immunologic studies as well as the relevant immunohistologic techniques employed for this study have been described previously in detail.[11]

Reagents

Details of the antibodies used in this study, their reactivity, and their sources are listed in Table 1. Biotinylated, affinity-purified anti-mouse antibody and avidin–biotin complex were obtained from Vector Laboratories, Burlingame, California. The substrate color reaction product was developed with 3-amino-9-ethylcarbazol (AEC)[12] obtained from Polyscience Inc., Warrington, Pennsylvania.

Immunogenetic Studies

High-molecular-weight DNA was prepared from frozen tissue sections by a method previously described elsewhere.[13] After digestion with appropriate restriction endonucleases, Southern blot hybridizations with nick-translated probes were used for analysis of the arrangements of immunoglobulin genes in the specimens. The IgH and TCRβ loci were analyzed after digestion of DNA with endonucleases Bam HI, Eco RI, or Hind III. The heavy-chain probe was a 6-kb fragment from the heavy chain joining region.[14] For analysis of TCRβ we used a 440bp Pst I-Hinc II constant region fragment from pJurkat-2.[15] Arrangements at the kappa locus were analyzed in Bam HI or Bgl II digests, using a 2.5Kb Eco RI constant region probe.[16] Those at the lambda locus were analyzed in Eco RI digests using a 5Kb Eco RI-Hind III constant

Table 1—Antibody Panel

Antibody	Predominant immunoreactivity	Source
Leu-1 (CD5)	T lymphocytes, some B-CLL	Beckton Dickinson
Leu-2a (CD8)	Suppressor/cytotoxic T lymphocytes	Beckton Dickinson
Leu-3a (CD4)	Helper T cells, some histiocytes	Beckton Dickinson
Leu-4 (CD4)	T lymphocytes	Beckton Dickinson
Leu-7 (NA)	Natural killer lymphocytes, some suppressor T cells	Beckton Dickinson
Leu-9 (CD)	T lymphocytes, some myeloid leukemias	Beckton Dickinson
Leu-11b (D16)	Natural killer lymphocytes	Beckton Dickinson
Leu-6 (CD1)	"Common" thymocytes, Langerhans cells	Beckton Dickinson
T3 (CD)	T lymphocytes	Coulter Immunology
T9	Cells bearing transferrin receptors	Coulter Immunology
T10	Immature thymocytes, plasma cells	Coulter Immunology
T11 (CD2)	T lymphocytes	Coulter Immunology
B1 (CD20)	B lymphocytes	Coulter Immunology
B2 (CD21)	B lymphocytes, dendritic reticulum cells	Coulter Immunology
B4 (CD)	B lymphocytes	Coulter Immunology
BA-1 (CD24)	Pre-B and B lymphocytes	Hybritech Inc.
BA-2 (CD9)	Cells in early stage of B-cell differentiation	Hybritech Inc.
J5 (CD10)	Common ALL antigen	Coulter Immunology
Sig, $\gamma,\mu,\delta,\alpha,\kappa,\lambda$	Immunoglobulin heavy and light chains	Tago Labs
I2(Ia)	HLA-DR related antigen	Coulter Immunology
Leu-M1 (CD15)	Monocytes, myeloid cells, some epithelial cells	Beckton Dickinson
TdT	ALL, some bone marrow cells, some epithelial cells	
Tac (CD25)	Activated T and B lymphocytes	Beckton Dickinson

NA, not applicable; CLL, chronic lymphocytic leukemia; ALL, acute lymphocytic leukemia.

region fragment.[17] Immunoglobulin gene probes were derived from the plasmids pHuJH, pHuKc, and pHuλc_2, a generous gift of Dr. P. Leder, Harvard Medical School. The pJurkat-2 plasmid was a gift of Dr. T. Mak, Ontario Cancer Institute. Control DNA was prepared from peripheral blood from a normal volunteer.

In order to determine the approximate proportion of cells carrying clonal gene rearrangements, scanning densitometry of autoradiograms was carried out on a Beckman Model DU-40 spectrophotometer equipped with a GelScan accessory and software (Beckman Instruments). Care was taken to choose films that were not overexposed. The maximum absorption was measured for each band, and the percentage rearranged was calculated by dividing the absorption of the rearranged band (or bands) by the sum of the rearranged and germline bands. These calculations provide an estimate of the maximum proportion of cells carrying a given rearrangement.

Results

Morphologic Findings

The morphologic findings in Case 1 were similar to those previously reported for lymphoblastic lymphoma from our laboratory.[18] The lymph node was diffusely infiltrated by a monomorphous population of immature mononuclear cells. The neoplastic lymphocytes had a fine nuclear chromatin pattern and a scanty cytoplasm with ill-defined cell borders. Mitotic figures were numerous. In Case 2, the lymph node architecture appeared to be partially intact; numerous reactive follicles present throughout the cortex were surrounded by a distinct mantle zone of small lymphocytes. The interfollicular areas, however, were expanded by a proliferation of atypical lymphocytes, plasma cells, and immunoblasts, consistent with a peripheral T-cell lymphoma of the diffuse mixed cell type. Histopathologic examination in Case 3 revealed a diffuse large cell lymphoma of the immunoblastic polymorphous type.

Immunologic Findings

Table 2 summarizes the antigenic phenotypes for our 3 cases. The neoplastic lymphoid cells in all 3 cases expressed several T-cell–associated antigens. Tumor cells from Cases 2 and 3 were negative for surface immunoglobulin (SIg) and for all of the B-cell–associated antigens evaluated. Neoplastic cells in Case 1 were also negative for SIg and for all of the B-cell–associated antigens except B2.

Immunogenetic Findings

The results of gene rearrangement analysis of all 3 cases are summarized in Table 3. Analysis of the T-cell receptor β-chain constant region genes in Bam HI and Hind III digests showed rearrangements in all 3 cases (Figure 1). The Eco RI digests showed deletion of the Cβ1 gene in Cases 1 and 2 and rearranged genes in Cases 2 and 3.

Table 2—Immunologic Data

Antigen	Case 1	Case 2	Case 3
Leu-1 (CD5)	+	+	+
Leu-2a (CD8)	+	−	−
Leu-3a (CD4)	+	+	+
Leu-4 (CD3)	ND	+	+
Leu-7	−	−	ND
Leu-9 (CD7)	ND	+	+
Leu-11 (CD16)	−	−	ND
Leu-6 (CD1)	−	−	−
T3 (CD3)	ND	+	+
T9	+	+	+
T10	−	−	−
T11 (CD2)	+	+	+
B1 (CD20)	−	−	−
B2 (CD21)	+	−	−
B4 (CD)	ND	−	−
BA-1 (CD24)	−	−	ND
BA-2 (CD9)	−	−	ND
J5 (CD10)	−	−	−
SIg	−	−	−
Ia	−	+	+
Leu-M1 (CD15)	−	−	−
TdT	+	−	ND
Tac (CD25)	−	+	−

+, reactive; −, nonreactive, ND, not done.

Table 3—Summary of Gene Rearrangements

	Ig heavy chain			Ig κ		Ig λ	T-cell receptor β-chain		
	B	E	H	B	Bg	E	B	E	H
Case 1	G	G	G	R	R	G	R	D	R
Case 2	G	R	G	R	R	G	R	G/D	R
Case 3	G	R	R	R	R	G	R	R	R

Genomic DNA from Cases 1, 2, or 3 were digested with the indicated restriction enzymes. B, Bam HI; E, Eco RI; H, Hind III; Bg, Bgl II. After transfer to nylon membranes, hybridizations were carried out as described. G, germline; R, rearranged, D, deleted.

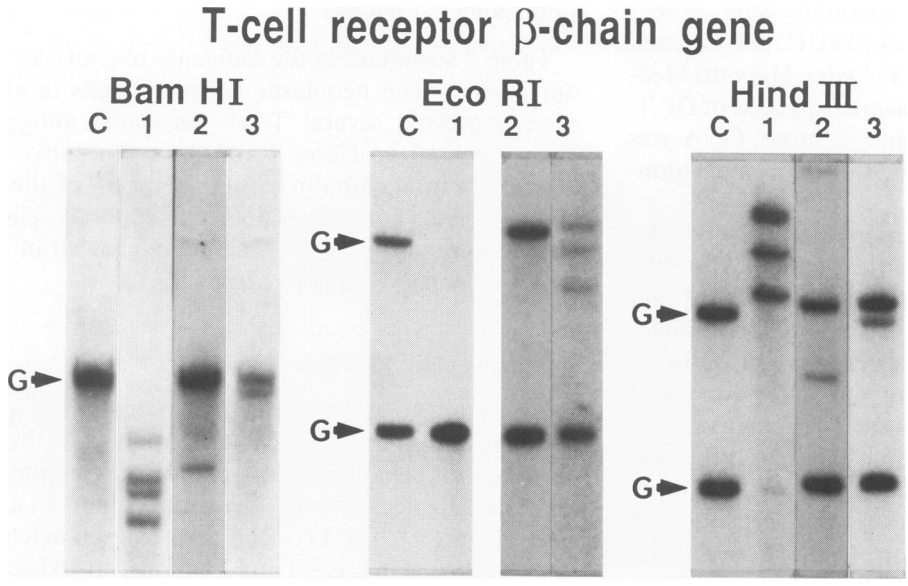

Figure 1—Analysis of DNA revealed rearrangement of TCRβ in all 3 cases. The position of the unrearranged germline bands is indicated by the letter G.

All 3 cases showed rearrangements of the immunoglobulin κ light chain gene in two different restriction digests (Figure 2). Cases 2 and 3 also displayed immunoglobulin heavy chain rearrangements (Table 3), but Case 1 clearly had only the germline configuration of the heavy chain gene when three different digests were probed (Figure 3). The λ light chain genes remained in the germline configuration in all 3 cases (Table 3).

Scanning densitometry was employed to obtain estimates of the relative fractions of cells carrying clonally rearranged genes in Cases 2 and 3, and the results are summarized in Table 4.

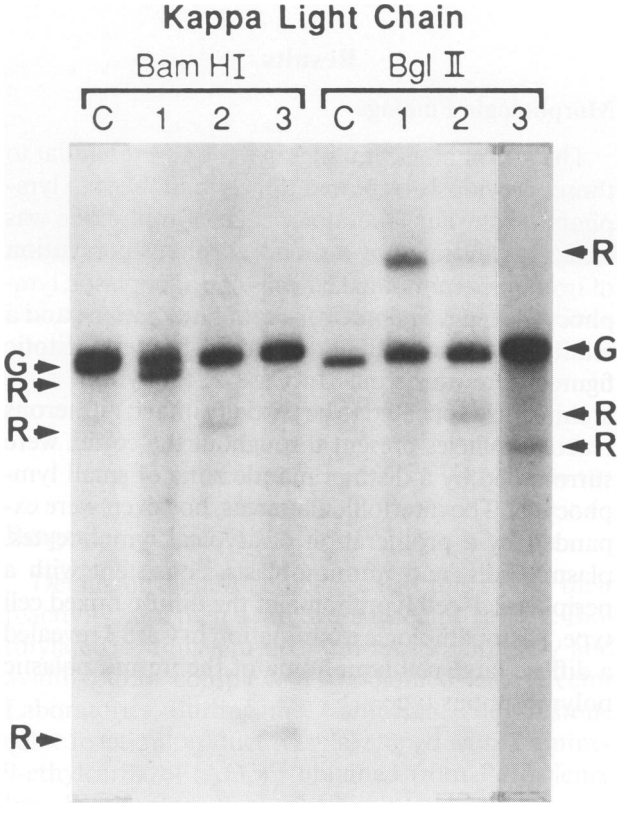

Figure 2—Analysis of light-chain immunoglobulin gene rearrangement revealed rearrangement of κ light chain genes in all 3 cases. Rearranged bands are indicated by the letter *R*.

Discussion

The application of molecular hybridization as a method complementary to conventional morphologic and immunologic studies has recently been shown to be helpful in the characterization of lymphoproliferative disorders (LPD).[1-10] It has been established that a strong correlation exists between the immunologic phenotype of individual neoplasms and the rearrangements of specific gene regions.[1-10] Moreover, because the DNA hybridization technique enables one to identify a clonal population of cells in as little as 1% of the total volume of tissue sampled,[3] the clonality of LPD can be established with this technique even in the absence of detectable immunologic markers.

Because rearrangement of immunoglobulin genes is an obligatory step for activation of these genes, they are rearranged in B-cell–derived neoplasms. However, it has been shown clearly that rearrangement of immunoglobulin heavy-chain genes is not limited to cells of B-cell ontogeny, but may also be seen in myeloid as well as T-cell processes.[2,7] Rearrangements at the immunoglobulin heavy-chain locus have been demonstrated in as many as 10% of T-cell leukemias.[8]

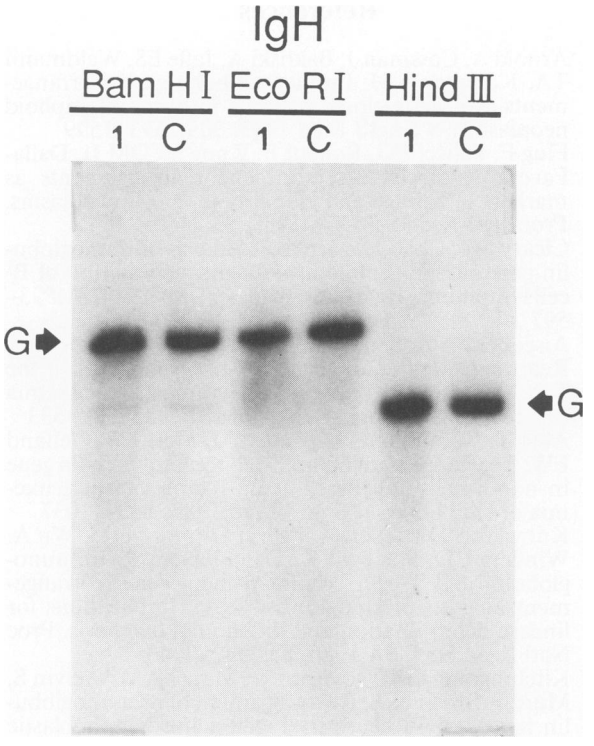

Figure 3—Analysis of the heavy chain gene configuration in Case 1 showed only the germline configuration.

Therefore, heavy-chain immunoglobulin gene rearrangements are not specific for B-cell lineage, and they alone do not conclusively establish the B-cell nature of LPD.

In contrast to B-cell neoplasms in which monotypic SIg serves as a reliable immunologic marker for the presence of clonal expansions of B cells, T cells do not have any markers of immunologic differentiation which identify T-cell clonality. For this reason, immunogenetic analyses of T-cell receptor gene rearrangement appeared to play a significant role in the determination of clonality in T-cell LPD.[2,4,8] However, similar to the rearranged heavy-chain immunoglobulin genes seen in occasional T-cell neoplasms, rearranged TCRβ genes have been observed in large series of B-cell lymphomas, and these may be seen in approximately 10% of B-lymphocytic leukemias.[8] The nonspecificity of heavy-chain immunoglobulin gene rearrangements in T-cell processes may be analogous to the presence of the TCRβ gene rearrangement in a B-cell neoplasm. Although rearrangements of immunoglobulin heavy-chain genes can be found in non-B-cell neoplasms, the identification of a somatic rearrangement of the light-chain immunoglobulin gene is extremely unusual in lymphoid lesions other than those derived from B cells. The identification of immunoglobulin light-chain gene rearrangements is considered to be a definitive indication of commitment to B-cell lineage.[3]

Table 4—Proportion of Cells With Gene Rearrangements

	B lineage (%)	T lineage (%)
Case 2	10	20
Case 3	6	31

The proportion of cells carrying clonal gene rearrangements was determined by scanning densitometry of autoradiograms. For determining the proportion of B lineage cells, one heavy chain lane and two lanes showing κ chain rearrangements (Bam HI and Bgl II) were traced and the results were averaged. For T-lineage determination, the Bam HI and Hind III lanes probed with TCRβ were scanned, and the results were averaged. In the Hind III lanes, only the germline 8kb Cβ2 constant region band and its products were included in the calculations.

In this report, we describe three NHLs that were classified immunologically as T-cell and in each of which molecular hybridization analysis showed rearrangement of the TCR β-chain gene, consistent with the presence of clonal populations of T cells. An unexpected finding, however, was the presence of rearrangements of the κ light chain locus in all three cases. Except in a single case report, rearrangements of light-chain genes have not been demonstrated in T-cell LPD. Ha-Kawa et al[19] recently reported on an unusual immunogenetic finding in a 10-year-old child with T-lymphoblastic lymphoma in whom analysis of DNA from the neoplastic cells not only revealed a TCRβ gene rearrangement but also showed rearrangements of the heavy-chain and κ light-chain genes.

An interesting observation on our Case 1 was the presence of a light-chain rearrangement in the absence of a heavy-chain rearrangement. Although the presence of a rearranged immunoglobulin light chain gene in the absence of a rearranged immunoglobulin heavy-chain gene has recently been reported,[20] the rearrangement of immunoglobulin genes almost always occurs in an ordered hierarchy, with light-chain gene rearrangements following heavy-chain gene rearrangements.[21] Therefore, B cells that have progressed to κ light-chain gene rearrangement should have had previous rearrangements of the heavy-chain genes. The finding of rearranged κ light-chain genes in the absence of rearranged heavy-chain genes, as encountered in our case, would appear to violate this rule of ordered Ig gene rearrangement and transcriptional regulation that has been demonstrated in studying B-cell–derived neoplasms.

An additional unusual result in Case 1 was the finding of four rearrangements of the TCRβ gene, instead of the expected maximum of two, one for each chromosome. This multiplicity can be accounted for in several ways. An aberrant rearrangement, such as unequal crossing over, or extra rearrangements in a subclone of malignant cells, such as further DJ or VDJ

recombination, could generate additional rearranged bands.[9] Alternatively, the malignant cells could be aneuploid; extra copies of Chromosome 7 (which carries the TCRβ gene) would account for the additional rearranged genes.[9] Finally, the neoplastic cells might be truly biclonal. We are presently unable to distinguish among these alternatives.

In Case 1, all the cells clearly carried rearranged TCRβ genes and κ light chain genes. It remains possible that the altered κ gene band might be due to chromosomal breakage, rather than conventional immunoglobulin gene rearrangement. In the absence of cytogenetic analysis, we can only speculate about this possibility.

The different intensities of the rearranged bands for T-cell receptor and κ light-chain genes in Cases 2 and 3 raise the possibility that the specimens contained separate clones of B and T cells. Densitometric analysis of autoradiograms (Table 4) suggests that this indeed could be the situation in Case 3, where B- versus T-cell specific rearrangements were carried by approximately 6% and 31% of the cells, respectively. Case 2, where the proportions of cells were approximately 10% and 20%, respectively, might or might not also represent a biclonal process, because these results may well be within the error of measurement of this technique.

In addition to the usual immunogenetic results, the immunologic findings were unexpected in Case 1. In Cases 2 and 3, the neoplastic cells expressed only T-cell–associated antigens. In Case 1, however, there was expression of a B-cell–associated antigen (B2) in addition to the presence of T-cell–associated antigens in neoplastic cells. The anti-B2 monoclonal antibody reacts with the C3d component of the complement receptor found on B cells and follicular dendritic reticulum cells.[22] Although rare cases of lymphoblastic lymphoma expressing B2 antigen have been reported,[23] in our experience this antigen is generally absent in T-cell–derived neoplasms.[24]

Based on the immunologic markers and on the presence of TCRβ gene rearrangements, we believe that our three NHLs should be classified as T-cell malignancies. However, the unusual presence of κ light-chain rearrangements in these cases is indicative of, at least in Case 1, lineage infidelity beyond that previously reported. Cases 2 and 3 may represent the same process or may be T-cell lymphomas that additionally contain monoclonal expansions of B cells. Our results indicate that although molecular-hybridization technology has proved helpful in the classification of LPD, the interpretation of molecular hybridization data requires correlation with morphologic and immunologic findings.

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