LYMPHOID TUMORS DISPLAYING REARRANGEMENTS OF BOTH IMMUNOGLOBULIN AND T CELL RECEPTOR GENES

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Lymphocytes characteristically express antigen recognition molecules; Ig in B cells, and T cell receptor(s) in T cells. A distinctive feature of the genetic loci coding for these molecules is that they must undergo a series of hierarchically ordered somatic rearrangements in order to become functionally active in mature lymphocytes (1–4). Ig gene rearrangements occur early during B cell differentiation (5), and are unique to each B cell clone, their diversity representing the genetic basis for antibody diversity (1, 2). Therefore, Ig gene rearrangements represent specific markers of B cell lineage, clonality, and differentiation for the study of normal and neoplastic B cell populations (5–7). The recent (8, 9) identification of the genetic locus coding for the β chain of the T cell receptor (T_β) has allowed the extension of this approach to the study of T cells. T_β gene rearrangements represent markers on T cell lineage and clonality, and have been proven to be similarly useful in identifying individual T cell clones (10–12). The availability of genetic markers of lineage and clonality for both B and T cells, and of monoclonal antibodies (mAb) that detect B and T cell–specific differentiation antigens allows a comprehensive analysis of lymphoid differentiation pathways, both in normal cell populations and in lymphoid tumors.

In this respect, a critical issue is the lineage specificity of Ig and T_β gene rearrangements in B and T cells, respectively. Although the overall specificity of Ig gene rearrangements in B cells has been clearly demonstrated, several studies (13–21) have reported occasional instances in which both normal and neoplastic T cells exhibit Ig gene rearrangements. Conversely, during our recent studies (12) aimed at establishing the specificity of T_β gene rearrangements in T cell neoplasms, we found B cell tumors in our control group that showed T_β gene rearrangements. These preliminary observations prompted us to directly address the issue of the respective B and T cell lineage specificity of Ig and T_β gene rearrangements in human lymphoid tumors.

[1] Abbreviations used in this paper: C, constant region of Ig; cDNA, complementary DNA; H, heavy chain of Ig; J, joining region of Ig; L, light chain of Ig; mAb, monoclonal antibody; T_β, β chain of T cell receptor for antigen; V, variable region of Ig.

We analyzed the patterns of rearrangement of Ig and T_β genes and the expression of mAb-defined B and T cell–associated differentiation antigens in a large panel of lymphoid neoplasms representative of the various clinicopathologic categories of human lymphoid neoplasia. We report that ~10% of lymphoid tumors, carrying either B or T cell–specific phenotypic markers are bigenotypic, i.e., display rearrangements of both Ig and T_β genes. The implications of these findings for both normal and neoplastic lymphoid differentiation are discussed.

Materials and Methods

Specimens.   Representative samples of lymph nodes, peripheral blood and bone marrow were collected during the course of standard diagnostic procedures. The diagnosis of each lymphoid neoplasm was established by conventional clinical, cytochemical and histopathological criteria. Assignment of the B and T cell lineage was based on results of analysis of cell surface markers (see below). A mononuclear cell suspension of >95% viability was prepared from each specimen by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation. The vast majority, >80%, of the mononuclear cells isolated from each specimen were cytomorphologically neoplastic.

Cell Marker Analysis.   The presence of cell surface Ig (sIg), cytoplasmic μ heavy chains (Cμ), sheep erythrocyte (E) rosette formation, HLA-DR antigens, common acute lymphoblastic leukemia antigen (CALLA), and terminal deoxynucleotidyl transferase (TdT) were determined as previously described (22). The expression of B and T cell–associated and –restricted differentiation antigens was determined by indirect immunofluorescence employing a panel of mAb (OKT3, OKT4, OKT6, OKT8, OKT10, OKT11, OKB2, BA-1, BA-2, B1, Leu-12, BL1, BL2, BL3, BL7) whose characteristics and distribution of reactivity have been previously determined (reviewed in 22). A cell population was considered positive if >25% of the neoplastic cells reacted with mAb.

DNA Extraction and Southern Blot Analysis.   DNA was prepared by cell lysis, proteinase K digestion, extraction with phenol, and precipitation with ethanol (23). 15 μg of DNA were digested with the appropriate restriction endonuclease, electrophoresed in a 0.8% agarose gel, denatured, neutralized, transferred to a nitrocellulose filter, and hybridized according to Southern (24). Filters were washed in 0.2 × standard sodium citrate, 0.5% sodium dodecyl sulfate, pH 7, at 60°C for 2 h (25).

DNA Probes.   The T_β probe used in this study was derived from a human complementary DNA (cDNA) T_β clone (YTJ-2) isolated from the Jurkat-2 T lymphoma cell line (a gift from Dr. T. Mak, University of Ontario Cancer Institute, Toronto, Canada). This clone hybridizes to both the alleles ($C_{\beta 1}$ and $C_{\beta 2}$) of the constant (C) region, and to one or more alleles of the variable (V) regions (10, 12). To generate a probe representative for the C region ($T_\beta C$), the insert of the YTJ-2 plasmid was digested with Hinc II restriction endonuclease, and the 0.8 kilobase (kb) T_βC-specific fragment was purified by preparative agarose gel electrophoresis. The Hinc II restriction site separates the V from the C regions by interrupting the joining (J) region (8, 10, 12). Probes for the Ig loci, representative of the J region (J_H) of the heavy chain (IgH) locus and of the constant k and λ regions (C_k and C_λ) of the light chain loci (IgL) were obtained from Dr. Stanley Korsmeyer (National Institutes of Health, Bethesda, MD), and have already been described (5, 6, 19). DNA fragments were ^{32}P-labelled by nick translation for use as probes (26).

Results

We analyzed a collection of 63 lymphoid malignancies, including 33 B cell and 30 T cell neoplasms, representative of virtually all the major clinicopathologic subtypes of B and T cell lymphomas and leukemias (Table I). DNA from these tumors were analyzed for Ig and T_β gene rearrangements by Southern blotting hybridization using various DNA clones representative of different portions of

TABLE I

IgH and T_β Gene Rearrangement in B and T Cell Neoplasms

Diagnoses*	Cases studied	Cases with rearrangements	
		IgH locus	T_β locus
B cell neoplasms			
ALL	11	11	3
NHL	12	12	1
CLL	3	3	1
MM	2	2	0
BL	5	5	0
Total:	33	33	5
T cell neoplasms			
ALL	4	1	4
NHL	3	0	3
PL	5	0	5
ATCLL	2	1	2
CTCL	2	0	2
SS	2	0	2
CLL	12	0	12
Total:	30	2	30

* ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin's lymphoma; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; BL, Burkitt's lymphoma; PL, prolymphocytic leukemia; ATCLL, acute T cell leukemia-lymphoma; CTCL, cutaneous T cell lymphoma; SS, Sezary syndrome.

Ig and T_β gene loci as probes. The IgH locus was studied by hybridization of Hind III– and Eco RI–digested DNA to a J_H-specific probe, the Ig k locus by hybridization of Bam HI–digested DNA to a C_k-specific probe, and the Igλ locus by hybridization of Eco RI– and Hind III–digested DNA to a C_λ-specific probe. For T_β gene analysis, DNA were digested with Bam HI and Eco RI, and hybridized to a probe that recognizes rearrangements of both ($C_{\beta1}$ and $C_{\beta2}$) the alleles of the T_β constant gene ($T_\beta C$; see Material and Methods).

Each of the 33 B cell neoplasms displayed Ig gene rearrangements (Table I). Some of these tumors displayed rearrangements of only the H chain locus, whereas others displayed rearrangements of both H and L chain loci (k or k plus λ; data not shown). Conversely, each of the 30 T cell neoplasms displayed $T_\beta C$ gene rearrangements. However, 7 cases displayed dual rearrangements, i.e. 5 of 33 B cell tumors (15%) displayed T_β gene rearrangements, and 2 of 30 T cell tumors (7%) displayed Ig gene rearrangements.

The pattern of Ig and T_β gene rearrangements of each of these seven cases is summarized in Table II, and representative data are illustrated in Fig. 1. We have also added to these data the analysis of the T_β gene variable region ($T_\beta V$) by hybridizing Eco RI–digested tumor DNA to a T_β probe that is representative of the C region and of one or more of the members of the presently undefined family of $T_\beta V$ genes. While a rearrangement detected by this probe demonstrates the involvement of this particular V region, the lack of a detectable rearrangement does not exclude the possibility of a rearrangement of a different V region.

TABLE II

Ig and T_β Gene Analysis and Immunophenotypic Profiles of Bigenotypic Tumors

Case	Diagnosis*	Tβ gene‡	Ig genes‡			T/T11	T3	T4	T6	T8	T11	TdT	Ia	CALLA	Cμ	sIg	Leu-12	BA2	BL2	OKB2	BL1	BA1	B1	BL3	BL7
			H	κ	λ																				
1	B-CLL	R	R	R	R	-	-	-	-	-	-	-	+	-	NT§	+	+	-	+	+	+	+	+	+	+
2	B-NHL	R	R	R	R	-	-	-	-	-	-	-	+	-	+	-	+	-	+	+	-	+	+	+	+
3	B-ALL	R	R	G	G	-	-	-	-	-	-	+	+	+	-	-	+	-	+	+	+	+	+	+	+
4	B-ALL	R	R	R	R	-	-	-	-	-	-	+	+	+	-	-	+	-	+	+	-	-	-	-	-
5	B-ALL	R	R	R	R	+	+	+	NT	+	-	+	+	+	-	-	+	+	-	-	+	+	-	-	NT
6	T-ALL	R	R	R	G	+	+	+	NT	+	NT	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	ATCLL	R	R	G	G	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* As in Table I.
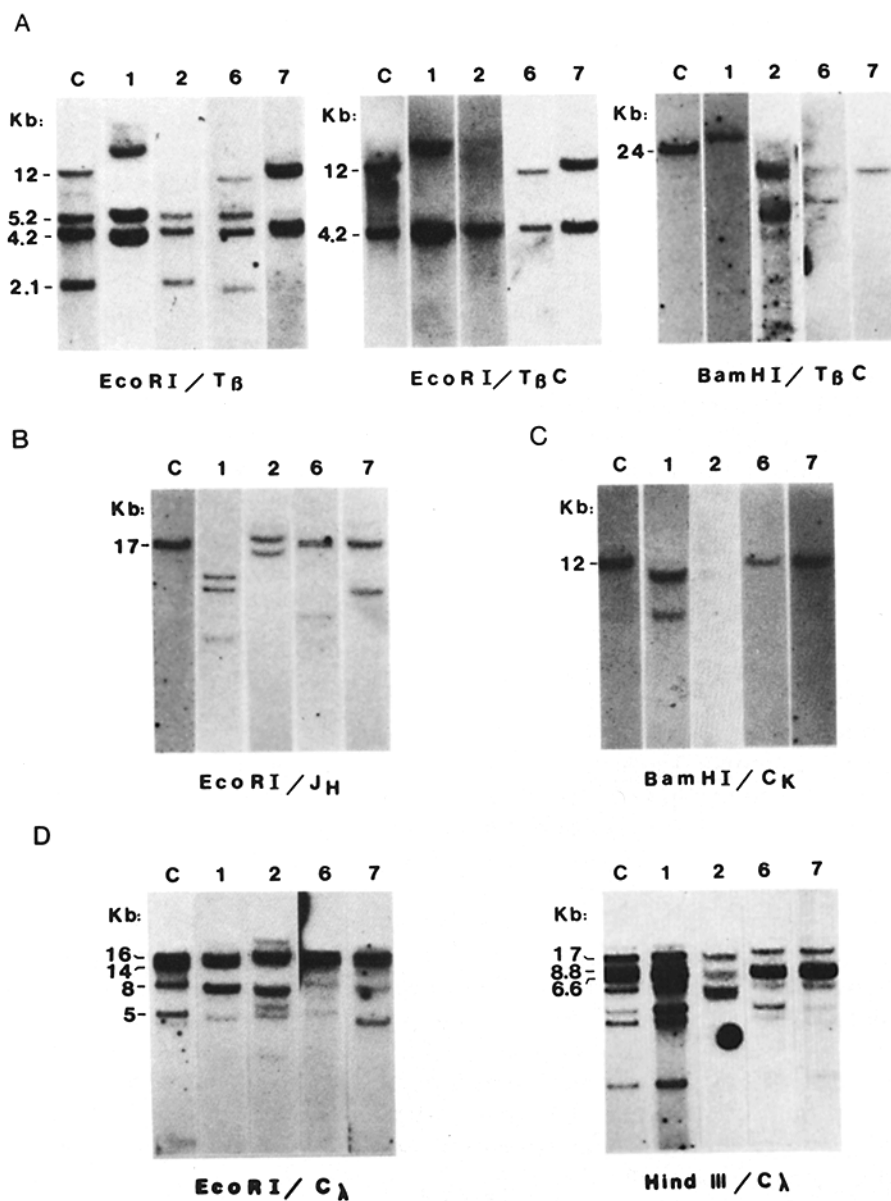‡ R, rearrangement and/or deletion. G, Germline configuration.
§ NT, not tested.

FIGURE 1. Ig and T_β gene rearrangements in bigenotypic lymphoid tumors. DNA from normal human fibroblasts (C) and from tumor cells (lanes 1, 2, 6, and 7 correspond to DNA from tumor cases in Table II) were analyzed for T_β (A), IgH (B), IgK (C) and Igλ (D) gene rearrangements using the restriction enzyme and DNA probe indicated at the bottom of each blot.

As outlined in Table II, two B cell tumors displayed rearrangements of both the C and V regions of the T_β gene, while three cases displayed rearrangements of only the $T_\beta C$ region. These five B cell neoplasms exhibited heterogeneity in their pattern of Ig gene rearrangements. Three cases (1, 2, and 4) had rearrange-

ments of all three Ig loci (see cases 1 and 2 illustrated in Fig. 1); case 5 displayed rearrangement of the H chain and K loci, and case 3 displayed rearrangement only of the H chain locus. Both T cell tumors displayed Ig gene rearrangements limited to the H chain locus (see Fig. 1); case 6 exhibited rearrangement of both C and V regions of the T_β gene, and case 7 exhibited rearrangement of the $T_\beta C$ region but no rearrangement of the particular V region explored by our probe.

Finally, we investigated whether the seven bigenotypic neoplasms had a corresponding biphenotype, i.e. expressed both B and T cell differentiation markers. We therefore extended our preliminary diagnostic immunophenotypic analysis of these seven cases to include analysis with our large panel of B or T specific mAb. As shown in Table II, not one of the five B cell neoplasms expressed any T cell–restricted antigens, and neither one of the two T cell neoplasms expressed any B cell–associated antigens. It is important to note that these neoplasms are representative of both the early and late stages of B and T cell differentiation. We conclude that bigenotypic lymphoid neoplasms displaying a mixed T and B genotype appear to retain B and T cell lineage–restricted cell surface antigens, and do not display a mixed immunophenotype.

Discussion

In the studies presented here, we report seven human lymphoid neoplasms that show rearrangements of both Ig and T_β genes. This group of bigenotypic lymphoid tumors is not irrelevant, since it represents ~11% of the malignant lymphoproliferative disorders that we investigated. The test group consisted of a heterogeneous group of 63 lymphoid neoplasms expressing diverse phenotypes consistent with various stages of differentiation in the B and T cell lineages, and representative of the majority of the significant clinical and histopathological subtypes of lymphoid neoplasia.

The bigenotype appears to be less frequent in T cell neoplasms, where it occurred in only 2 of 30 of the cases (7%) studied. The frequency of Ig gene rearrangements in freshly isolated T cell neoplasms in this series, 7%, is significantly lower than the 50% frequency rate previously reported (13, 15, 18, 19) to occur in T cell lymphoid cell lines in which, although not demonstrated, the presence of T_β gene rearrangements can be reasonably assumed. Our data establish the low frequency of Ig gene rearrangement in primary cell tumors, and suggest that similar rearrangements may frequently occur and/or be selected for during continuous cell culture.

The occurrence of the mixed genotype appears to be approximately twice as frequent in B cell than in T cell neoplasms, since it occurred in five out of 33 (15%) of the cases in this series. The difference between the frequency of combined rearrangements in B and T cell tumors appears to correlate with qualitative differences in the pattern of gene rearrangements. In fact, Ig gene rearrangements that occur in T cell tumors have been limited to the H chain locus in all cases. These rearrangements most likely reflect a partial D-J joining event, as demonstrated in a number of human and murine T cell lines (14). Conversely, our data indicate that the T_β gene rearrangements that occur in B cell tumors may involve both C and V regions, indicating that a full V-D-J joining

event may have occurred. Insights into the significance of this difference will be provided by studies of specific mRNA and protein expression in these cells.

Our findings in tumor cells raise the question of whether combined Ig and T_β rearrangements represent a consequence of neoplastic transformation, or can occur during normal lymphoid differentiation. In the former case, it is possible that the dual genotype is a transformation-related event that reflects the derangement of the genetic machinery regulating differentiation in the neoplastic cells. Thus, these bigenotypic lymphoid neoplasms would represent examples of lineage infidelity within the lymphoid lineage comparable to what has been observed between the lymphoid and the myeloid lineages (27). However, these bigenotypic lymphoid neoplasms retain a completely fidelous immunophenotypic pattern, while the observed cases of lymphoid-myeloid lineage infidelity are biphenotypic. An additional, attractive possibility is that these unorthodox rearrangements may involve oncogene sequences contributing to the pathogenesis of these tumors (28–31).

Alternatively, the dual genotype may also occur in normal cells, as strongly suggested by several studies (16, 17) reporting the occurrence of Ig H chain locus rearrangements and expression in mouse thymocytes and lymphocytes. If this is the case, then several interpretations can be proposed to attempt to explain the significance of this phenomenon. (a) This event may have some functional significance, and may identify a subpopulation of bipotent lymphoid cells, i.e. with both B and T cell properties. We consider this theoretical possibility rather unlikely in view of our observation that each of our bigenotypic neoplasms retained a pure B or T cell phenotype. (b) Dual genotypes may reflect the existence of a differentiative step during lymphoid differentiation in which a committed B or T cell retains the potential to switch to the other lineage. The fidelity of the immunophenotype may represent the final lineage commitment of the cell, whereas the dual genotypic markers remain as an irreversible footprint of the previous commitment. (c) The dual genotype may reflect the concomitant, perhaps casual, activation in T and B cells of common molecular mechanisms, regulating the rearrangements of these loci. This notion is consistent with the reported (4) partial homology between sequences involved in the joining events in T_β and Ig H chain genes.

Finally, our findings bear important implications for the immunodiagnosis and classification of human lymphoproliferative malignancies. It has been proposed that Southern blot analysis and demonstration of Ig and T_β gene rearrangements represents the definitive method by which to determine the lineage and the clonality of lymphoid populations that display equivocal immunophenotypic patterns. However, our data presented here strongly suggests that the lineage specificity of these rearrangements is not absolute. We conclude that only a multiparametric approach (22), employing immunophenotypic, genotypic, and in some instances functional testing, allows a conclusive lineage definition of all lymphoid neoplasms.

Summary

Ig and T_β gene rearrangements can be used as genetic markers of lineage and clonality in the study of B and T cell populations. We have addressed the issue

of the respective B and T lineage specificity of these rearrangements by analyzing a panel of 63 lymphoid tumors representative of the various clinicopathologic categories of both B and T neoplasias. We report that ~10% of the cases tested displayed rearrangements of both Ig and T_β genes. Despite their dual genotypic pattern, these tumors retain a pure immunophenotype, i.e. they display either B or T cell lineage–restricted cell surface antigens. The implications of these findings for both normal and neoplastic lymphoid differentiation are discussed.

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