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T-cell receptor gene rearrangements as markers of lineage and clonality in T-cell neoplasms

(DNA rearrangements/T cells/T-cell neoplasm)

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ABSTRACT **Ig gene rearrangements represent markers of lineage, clonality, and differentiation of B cells, allowing a molecular diagnosis and immunogenotypic classification of B-cell neoplasms. We sought to apply a similar approach to the study of T-cell populations by analyzing rearrangements of the T-cell receptor β-chain (T_β) gene. Our analysis, by Southern blotting hybridization using T_β-specific probes of DNAs from polyclonal T cells and from 12 T-cell tumors, indicates that T_β gene rearrangement patterns can be used as markers of (*i*) lineage, allowing the identification of polyclonal T-cell populations, and (*ii*) clonality, allowing the detection of monoclonal T-cell tumors. In addition, our data indicate that T_β gene rearrangements represent early and general markers of T-cell differentiation since they are detectable in histologically different tumors at all stages of T-cell development. The ability to determine lineage, clonality, and stage of differentiation has significant implications for future experimental and clinical studies on normal and neoplastic T cells.**

A genetic approach involving the molecular analysis of Ig gene rearrangements has been used for the study and classification of B-cell malignancies (1–3). This approach is based on three fundamental properties of Ig gene rearrangements. First, they represent a specific and early event for B-cell differentiation and thus provide an irreversible genetic marker for B-cell commitment (4, 5). Second, a particular Ig gene rearrangement is specific for a given B-cell clone, allowing early and unequivocal identification of a clonal B-cell population (1–3). Third, Ig gene rearrangements follow a hierarchical pattern (i.e., heavy chain gene rearrangement precedes light chain gene rearrangement, with the κ gene preceding the λ gene) during B-cell development, allowing study and classification of B-cell neoplasms according to their stage of differentiation (refs. 6 and 7). On the basis of these concepts, the immunogenotypic analysis of B-cell neoplasms, using specific Ig DNA probes, is becoming a critical addition to surface marker and morphologic analysis in determining the lineage, clonality, and stage of differentiation of B-lymphocytic proliferation.

The functional and structural analogies between the antigen recognizing molecules of B lymphocytes—i.e., Igs—and that recognizing molecules of T lymphocytes—i.e., T-cell receptor(s)—suggest that extension of the immunogenotypic analysis to the study of T-cell proliferation may be possible. The recent isolation and characterization of DNA clones corresponding to the human T-cell receptor genes (8) has confirmed that, at the genetic level, Ig and T-cell receptor genes share several structural and functional features (9). In particular, analysis of T-cell receptor β-chain (T_β; it will be proposed at the Eighth International Workshop on Human Gene Mapping in Aug. 1985 that this gene be given the symbol *TRBC*) DNA clones has identified variable (*V*), diversity (*D*), joining (*J*), and constant (*C*) regions similar in size and sequence to the corresponding Ig elements. These segments are rearranged during T-cell differentiation by mechanisms that are likely to be analogous to those of the Ig genes (9–16).

Although many aspects of the molecular genetics of T-cell receptor genes are still unknown, the availability of a human T_β gene probe, together with some preliminary evidence of rearrangements in T-cell neoplasms (17), have prompted us to explore the possibility of a molecular study and classification of T-cell neoplasms based on T_β gene rearrangements. This possibility is especially intriguing in view of the marked clinical, morphological, and immunophenotypic heterogeneity of neoplastic T-cell proliferation and the current lack of an easily generated marker of clonality for T cells. In this report, we provide the experimental framework for using T_β gene rearrangements as markers of T-cell lineage and of clonality to identify monoclonal and polyclonal T-cell populations.

MATERIALS AND METHODS

Specimens. Representative samples of lymph nodes, thymus, a variety of normal nonlymphoid tissues, peripheral blood, and bone marrow were collected during the course of standard diagnostic procedures. The diagnosis was established in each case by standard clinical, histochemical, and histologic criteria and with the help of cell marker analysis (see Table 1 for diagnosis of T-cell neoplasms). A mononuclear cell suspension of >95% viability was prepared from each lymphoid tissue, peripheral blood, and bone marrow sample by Ficoll-Hypaque (Pharmacia) density gradient centrifugation. In the tumor cases tested, the majority, >80%, of the mononuclear cells isolated were cytomorphologically neoplastic.

Cell Marker Analysis. The presence of cell surface and cytoplasmic immunoglobulin, spontaneous sheep erythrocyte (E) rosette formation, and terminal deoxynucleotidyltransferase, and the expression of a variety of B- and T-cell-associated differentiation antigens, OKT3, OKT4, OKT6, OKT8, OKT10, BL1, BL2, BL3, BA-1, BA-2, B1, OKB1, OKB2, OKB4, and OKB7 (18), were determined as described (18). A cell population was considered positive if >25% of the neoplastic cells in a given neoplasm reacted with the antibody.

E Rosette Fractionation. In selected instances, purified fractions of E^+ (T) and E^- (non-T) cells were obtained by fractionation of peripheral blood mononuclear cells on a Ficoll-Hypaque density gradient according to their capacity to form E rosettes with *Vibrio cholerae* neuraminidase-

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Abbreviations: T_β, gene encoding the T-cell receptor β chain; *V*, *D*, *J*, and *C* region, variable, diversity, joining, and constant region; E, erythrocyte; kb, kilobase(s).

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treated sheep erythrocytes (18). The E^+ fraction consisted of >95% T cells as determined by expression of OKT3 and absence of B1. The E^- fraction consisted of <5% T cells as determined by OKT3 expression.

DNA Extraction and Southern Blot Analysis. DNA was prepared by cell lysis, proteinase K digestion, extraction with phenol, and precipitation with ethanol (19). Fifteen micrograms of DNA was digested with the appropriate restriction endonuclease, electrophoresed in an 0.8% agarose gel, denatured, neutralized, and transferred to a nitrocellulose filter, and hybridized according to established procedures (19). Filters were washed with 30 mM NaCl/3.0 mM Na citrate/0.5% $NaDodSO_4$, pH 7, at 60°C for 2 hr.

DNA Probes. The T_β probe uscd was derived from a human cDNA T_β clone (YTJ-2) isolated from the Jurkat-2 T-lymphoma cell line (8) and provided to us by Tak Mak. To generate probes representative for the *V* and *C* regions, the insert of the YTJ-2 plasmid was digested with *Hin*cII and the two resulting fragments were purified by preparative agarose gel electrophoresis. The *Hin*cII restriction site separates the *V* region from the *C* region by interrupting the *J* region (8, 17). For use as probes, DNA fragments were ^{32}P-labeled by nick-translation (19).

RESULTS

Germ Line Restriction Enzyme Patterns of the T_β Gene. We have determined the germ line restriction enzyme pattern of the T_β gene by using restriction endonucleases that do not display any genetic polymorphism in this locus to avoid the problem of distinguishing restriction enzyme polymorphisms from T_β gene rearrangements. A panel of 31 DNAs extracted from normal tissues and nonhematopoietic tumors from 31 individuals was digested with *Eco*RI, *Bam*HI, and *Hin*dIII restriction endonucleases and then analyzed by Southern blot hybridization using T_β DNA probes. Digestion with *Eco*RI revealed four hybridization bands in all control DNAs tested using the total T_β probe (Fig. 1). Separate hybridizations to the two subclones identified two *Eco*RI bands [12.0 and 4.2 kilobases (kb)] derived from the *C* region (25) and two bands (5.2 and 2.1 kb) derived from the *V* region. No evidence of polymorphism for the *Eco*RI restriction sites was found in the population tested. Digestion with *Bam*HI revealed a nonpolymorphic 24.0-kb band hybridizing to the *C* region probe, whereas two allelic variants were detected in our control population using the *V* region probe. A three-fragment pattern (7.5, 3.4, and 2.2 kb) was detected in the majority of cases. The second variant, lacking the 2.2-kb fragment, was identified in ≈30% of cases. *Hin*dIII restriction enzyme digestion and hybridization with the *C* region probe revealed two consistent fragment patterns (Fig. 1). The more frequent pattern, detected in 27 cases, is represented by an 8.7-kb fragment and a 3.8-kb fragment, while 4 cases revealed an additional 14.5-kb fragment. Hybridization with the *V* region probe consistently revealed a single nonpolymorphic 17.0-kb fragment.

In summary, our results confirm preliminary published information indicating the absence of *Eco*RI and *Bam*HI site polymorphisms in the *C* region (17) and identify a relatively rare *Hin*dIII allelic variant of the *C* region, which was previously reported as being nonpolymorphic for this enzyme (17). Based on these observations, *Eco*RI digestion was routinely used to identify DNA rearrangements of the T_β locus, and digestion with *Bam*HI and *Hin*dIII was used for additional analysis of the *C* and *V* regions, respectively.

Identification of a Marker of Lineage in T Cells. One objective of this study was to determine whether specific T_β gene rearrangements can be used as markers of clonality in T cells in the way that Ig gene rearrangements are used in B cells. For this purpose, we first analyzed the restriction enzyme pattern of the T_β gene in polyclonal T cells—namely, thymocytes and normal sheep erythrocyte rosette-positive (E^+) T lymphocytes from peripheral blood.

No rearranged bands were detected when E^+ cells or thymocytes were digested with *Hin*dIII, *Bam*HI, or *Eco*RI and hybridized to total, *C* region, or *V* region T_β probes. However, the *Hin*dIII and *Bam*HI patterns were indistinguishable from the established germ line pattern seen in rosette-negative (E^-) non-T lymphocytes, whereas consistent deletion of the 12.0-kb fragment corresponding to a portion of the T_β *C* region was detectable in both E^+ lymphocyte and thymocyte DNAs digested with *Eco*RI (Fig. 2). These findings, together with the fact that the T_β gene is rearranged in individual T-cell clones (see below and ref. 17), suggest that, although multiple individual rearrangements cannot be detected in a polyclonal population, the absence of the *Eco*RI 12.0-kb fragment may reflect a bi-allelic event common to all rearrangements in all T cells. We conclude that deletion of the *Eco*RI 12.0-kb band is a marker for the T-cell lineage and allows the identification of polyclonal T-cell populations.

T_β Gene Rearrangements as a Marker for T-Cell Clonality. We next investigated the occurrence of T_β gene rearrangements in T-cell neoplasms to evaluate the possible use of these rearrangements as markers of both lineage and clonality in monoclonal T-cell populations (Table 1). Twelve cases, representative of a spectrum of T-cell neoplasms, were analyzed. In addition to their different diagnostic features, these cases exhibited considerable immunophenotypic heterogeneity, indicating their derivation from cells blocked at

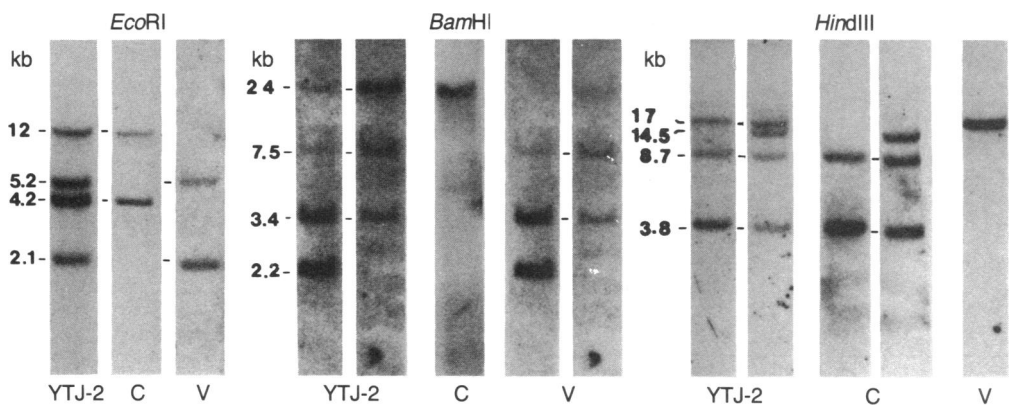

FIG. 1. Germ line restriction enzyme patterns of the T_β gene after digestion with *Eco*RI, *Bam*HI, or *Hin*dIII and hybridization with total (YTJ-2), *C* region, and *V* region probes. Representative patterns are shown for nonpolymorphic and polymorphic variants.

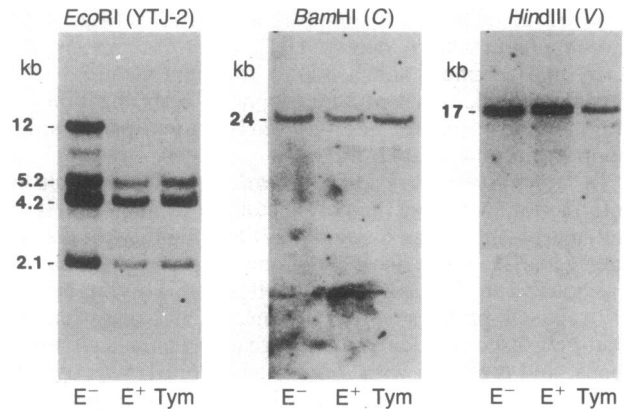

FIG. 2. T_β gene restriction enzyme patterns in normal peripheral blood non-T cells (E^-), normal peripheral blood T cells (E^+), and normal thymocytes (Tym). DNAs were digested with *Eco*RI, *Bam*HI, and *Hin*dIII and hybridized with total (YTJ-2), *C* region, and *V* region probes.

different levels of T-cell maturation. The T-lineage specificity of the T_β gene rearrangements was further assessed by including 33 cases of non-T hematopoietic malignancies in the analysis.

Using the scheme of restriction enzyme digestions and probes described above, we detected rearrangements in 11 of the 12 T-cell tumor DNAs tested. The complete analysis of four representative cases is illustrated in Fig. 3, and data relative to analysis of the remaining cases are summarized in Table 1. It is important to note that for some cases it was necessary to use more than one enzyme to document the presence of a rearrangement that was not detectable by *Eco*RI digestion alone (see Fig. 3).

All the cases displaying rearrangements also displayed deletion of the *Eco*RI 12.0-kb band, confirming the T-cell lineage specificity of this event. No T_β gene rearrangement or deletion was detectable in the 33 non-T hematopoietic neoplasms tested, with the exception of three B-cell derived acute lymphocytic leukemia cases, which displayed a rearranged T_β gene pattern. Although there are cases representing exceptions, which will be further discussed below and in future studies, we conclude that T_β gene rearrangements generally fit criteria of tissue and case specificity that allow their use as markers of lineage and clonality in T-cell neoplasms.

Different Patterns of T_β Gene Rearrangements in T-Cell Neoplasms. Detailed analysis of T_β gene rearrangements in T-cell tumors shows heterogeneity in the number of alleles and T_β gene regions involved. With respect to rearrangement involving the *C* region, *Eco*RI digestions indicate that, in all the tumors tested, rearrangements and/or deletions involve both alleles corresponding to the 12.0-kb fragment, whereas at least one allele of the 4.2-kb fragment is maintained in its germ line configuration (Fig. 3 and Table 1).

With respect to the *V* region we observed that the 12 T-lymphoma cases can be divided into two subgroups (Table 1). A first group is represented by 4 cases that display a rearranged *C* region and a germ line *V* region (e.g., cases 2 and 4 in Fig. 3). The second group is represented by 7 cases displaying rearrangements of both the *C* and the *V* regions. No case displaying a germ line *C* region and a rearranged *V* region was found (Table 1). These data suggest an order of events involving the rearrangement/deletion of at least part of the *C* region in both alleles, eventually followed by rearrangement of the *V* region(s). Alternatively, it is possible that rearrangement and expression of *V* regions may not occur in some T-cell subpopulations. These preliminary observations are limited by the fact that our probes cannot explore the precise mechanisms of rearrangements (e.g., *V–D–J* joining events) and by the fact that our *V* region probe may not recognize the entire region, leading to underestimation of the number of *V* region rearranged cases. Finally, all possible combinations involving deletion and/or rearrangement of one or both the *V* region alleles were observed (Table 1).

T_β Gene Rearrangements Occur Early During T-Cell Differentiation. Next, we correlated the presence of T_β gene rearrangements with the diverse histological and immunophenotypic characteristics of our T-cell neoplasms (Table 1). The following three points can be derived from our preliminary survey. (*i*) The presence of T_β gene rearrangements is seen in virtually all T-cell neoplasms tested. (*ii*) T_β gene rearrangements are present in all of the functionally different T-cell populations as identified by the T4 (helper) and T8 (suppressor) markers and by functional assays (data not shown, ref. 17, and unpublished work). (*iii*) T_β gene rearrangements are present in clinically and histopathologically heterogenous T-cell tumors representative of almost the

Table 1. Diagnostic, immunogenotypic, and immunophenotypic features of T-cell neoplasms

		T_β gene rearrangement patterns				Presence or absence of phenotypic marker						
		*Eco*RI										
Case	Diagnosis	*C*	*V*	*Bam*HI	*Hin*dIII	E/T11	T3	T4	T6	T8	T10	TdT
1	T-CLL	C1, C2	V1, V2	C	V	+	−	−	−	+	−	−
2	T-CLL	C1*, C2, 1R	V1, V2	C*, 2R	V	+	+	+	−	+	−	−
3	SS	C1*, C2	V1, V2	C*, 1R	V	+	+	+	−	−	−	−
4	SS	C1*, C2, 1R	V1, V2	C*, 2R	V	+	+	+	−	−	−	−
5	ATCLL	C1*, C2, 1R	V1, V2	C*, 2R	V	+	+	+	−	−	+	−
6	ATCLL	C1*, C2, 1R	V1, V2	C*, 2R	V*, 1R	−	+	+	−	−	−	−
7	CTCL	C1*, C2, 1R	V1, V2, 1R	C, 2R	V, 1R	+	−	+	−	−	+	−
8	T-NHL	C1*, C2	V1*, V2*	C*, 2R	V*	+	+	−	−	−	+	+
9	T-NHL	C1*, C2	V1*, V2*	C*, 1R	V*	+	+	+	−	−	−	−
10	T-ALL	C1*, C2, 1R	V1*, V2*, 1R	C*, 2R	V, 1R	+	−	+	+	+	+	+
11	T-ALL	C1*, C2, 1R	V1, V2*	C*, 1R	V*, 1R	+	ND	+	ND	+	ND	+
12	T-ALL	C1*, C2	V1*, V2*, 1R	C*, 1R	V*, 2R	+	+	+	ND	+	+	+

T-CLL, T-cell chronic lymphocytic leukemia; SS, Sezary syndrome; ATCLL, adult T-cell leukemia/lymphoma syndrome; CTCL, cutaneous T-cell lymphoma; T-NHL, T-cell non-Hodgkin lymphoma; T-ALL, T-cell acute lymphocytic leukemia. ND, not determined. C1 and C2 refer, respectively, to the 12.0- and 4.2-kb *Eco*RI bands detected by the *C* region probe. V1 and V2 refer, respectively, to the 5.2- and 2.1-kb *Eco*RI bands detected by the *V* region probe. C and V refer to the single bands detected by the *C* and *V* region probes with *Bam*HI and *Hin*dIII, respectively. Asterisks indicate deletion of the corresponding band. R indicates number of rearrangement bands seen.

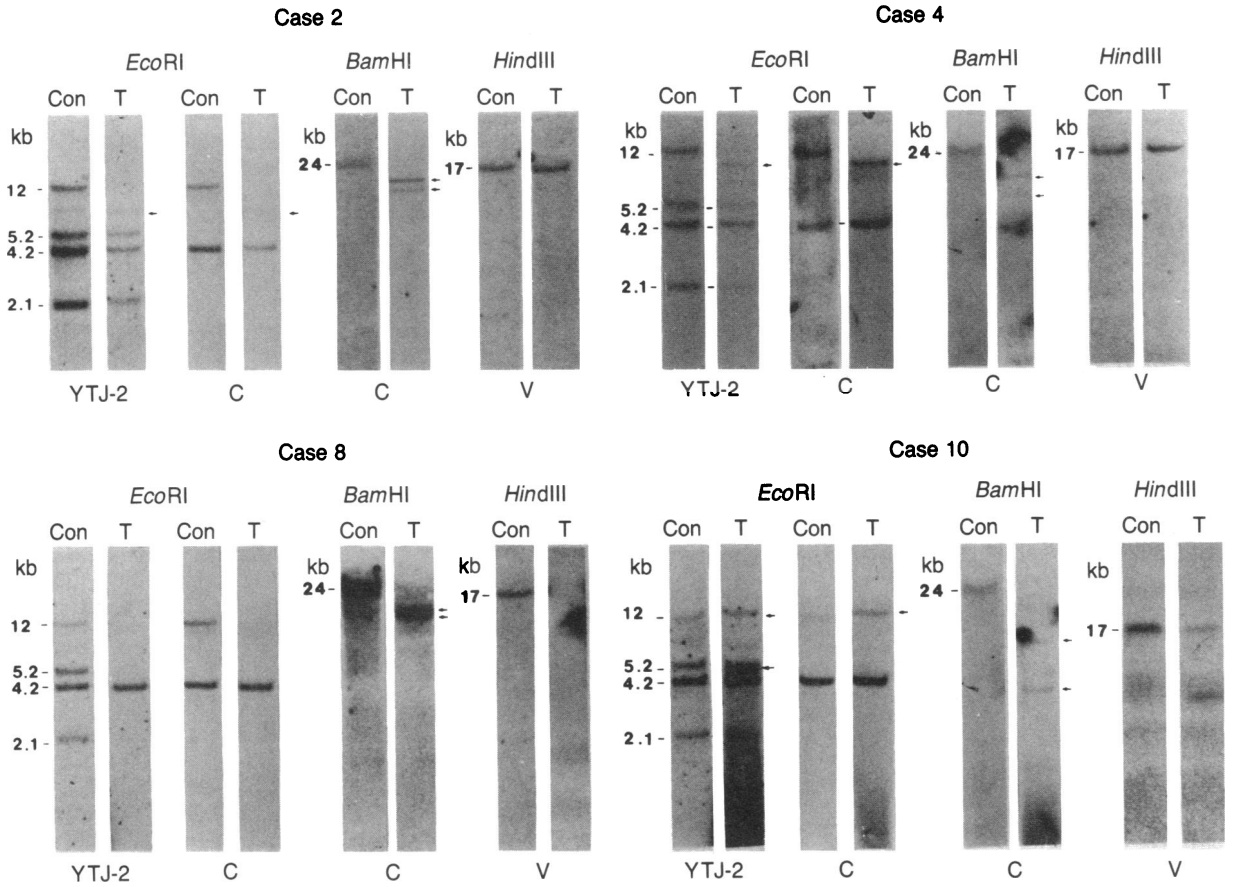

FIG. 3. T_β gene rearrangements in T-cell neoplasms. Case identification numbers correspond to those listed in Table 1. Restriction endonucleases and hybridization probes used are indicated. Four representative cases are shown. Cases 2 and 4 show rearrangement and/or deletion of *C* regions and the germ line *V* region, while cases 8 and 10 show rearrangements and/or deletions of both *C* and *V* regions. Con, germ line control. Dashes between lanes indicate conserved germ line bands. Arrows indicate rearrangement bands.

entire spectrum of T-cell neoplasia. Taken together, these data indicate that T_β gene rearrangements represent an early and irreversible marker of thymic maturation that can then be detected along different T-cell differentiation pathways.

DISCUSSION

Studies in which Ig gene rearrangements were used as markers of lineage, clonality, and differentiation in B-cell neoplasia have provided the rationale for attempting a molecular immunogenotypic analysis of T-cell populations based on T-cell receptor gene rearrangements. The still-preliminary characterization of the T_β gene and the lack of probes corresponding to sequences coding for other functional portions of the complete T-cell receptor prevent thorough understanding of all the molecular events involved in expression of the T-cell receptor gene. Nonetheless, our data provide a framework for using T_β gene rearrangements for analysis of normal and neoplastic T-cell proliferation.

Analysis of polyclonal populations of T lymphocytes and thymocytes showed that the disappearance of a 12.0-kb *Eco*RI fragment corresponding to a part of the *C* region of the T_β gene is a consistent marker of lineage that can be used to identify polyclonal T-cell populations. It is reasonable to assume that the disappearance of the *Eco*RI band may reflect a deletion/rearrangement event affecting both of the T_β region alleles. That this deletion/rearrangement event is not detectable by *Bam*HI digestion can be explained by the fact that the fragment involved may not be large enough to detectably affect the migration of the 24-kb *Bam*HI fragment containing the *C* region. Although further studies are needed to confirm this observation, we conclude that, from an operative point of view, analysis by *Eco*RI digestion is a valid tool to distinguish a polyclonal T-cell population from a monoclonal (i.e., displaying the deletion plus rearrangements) one or from a non-T-cell (i.e., displaying the germ line pattern) one.

Although the deletion of the *Eco*RI fragment identifies the T-cell lineage of a given lymphocyte population, the detection of rearrangements represents a marker of clonality. This criterion is supported by the finding that T_β gene rearrangements are usually detectable in T-cell neoplasms but not in non-T-cell tumors. In this respect, T_β gene rearrangements represent the counterparts, in T-cell tumors, of Ig gene rearrangements in B-cell tumors. Exceptions to this scheme can be found in both groups. The three B-cell tumors detected in this study that display T_β gene rearrangement may represent examples of lineage infidelity since they concomitantly display B-cell phenotypic markers, Ig gene rearrangements, and T_β gene rearrangement (unpublished work). More questionable is the nature of the T-cell tumor that shows neither T_β gene deletion or rearrangement nor Ig gene rearrangement yet displays some T-cell phenotypic markers. This case and analogous ones require further investigation because they may represent tumors arising from cells either blocked at a stage of T-cell differentiation preceding the appearance of T_β gene rearrangement or a currently unidentified T-cell subset not requiring expression of the β-chain of the T-cell receptor. However, the preliminary evidence that rearrangements of the genes coding for related, non-β, T-cell receptor chains may occur late in the T-cell differentiation pathway (9), together with the very high percentage of T-cell tumors displaying T_β rearrangements in our study, suggests that the T_β gene rearranges early during thymic development and that

rearrangements of this gene are early and general markers of lineage and clonality in these tumors.

These last observations are further supported by our data correlating the presence of various differentiation and subset-specific T-cell markers with the presence of T_β gene rearrangements. It has recently been reported that T_β gene rearrangement precedes the appearance of the OKT3 reactivity in thymocytes, assigning the appearance of rearrangements to stage II of thymic differentiation (20). Our data substantially confirm the precocity of T_β gene rearrangements since they are detectable in some OKT3-negative tumor cases. However, immunophenotypic analysis of T-cell neoplasms by us, as well as by other investigators (21), indicates that most T-cell neoplasms cannot be readily assigned to a specific maturation step since they often display an uncoupling in the expression of certain phenotypic markers. Thus, analyses of large collections of tumors are needed to definitively establish the criteria for a combined immunogenotypic/immunophenotypic classification of differentiation stages of T-cell neoplasms.

In conclusion, the use of analysis of rearrangement of the T-cell receptor gene to determine the lineage, clonality, and stage of differentiation of T-cell proliferation paves the way for other investigations of clinical and biological relevance.

A number of questions related to the clonality of certain chronic T-cell proliferative states (e.g., T-CLL) and to the different cellular components involved in some diverse lymphoid proliferations (e.g., Hodgkin disease) can now be effectively addressed. Finally, it has recently been shown that Ig gene loci play a role in specific chromosomal recombinations involving cellular oncogene loci in B-cell tumors (22–24) and the T_β gene rearrangements may play a similar role in development of T-cell neoplasms.

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