Recombinative Events of the T cell Antigen Receptor δ Gene in Peripheral T cell Lymphomas

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Abstract

Recombinative events of the T cell antigen receptor (TCR) δ-chain gene were studied in 37 cases of peripheral T cell lymphoma (PTCL) and related to their clinical presentation and the expression of the αβ or γδ heterodimers as determined by immunostaining of frozen tissue samples. There were 22 cases of αβ, 5 cases of γδ, and 10 cases of silent TCR expressing neither the αβ nor γδ TCR. 5 different probes were used to examine the δ locus. The 22 cases of αβ PTCL displayed biallelic and monoallelic deletions; a monoallelic Vδ1 Jδ1 rearrangement was observed in 1 case and a monoallelic germ line configuration in 7 cases. The 5 cases of γδ PTCL displayed biallelic rearrangements: the productive rearrangements could be ascribed to Vδ1Jδ1 joining in 3 cases and VJδ1 joining in 2 cases according to the combined pattern of DNA hybridization with the appropriate probes and of cell reactivity with the TCRδ-1, δTCS-1, and anti-Vδ2 monoclonal antibodies. In the VJδ1 joining, the rearranged V segments were located between Vδ1 and Vδ2. Interestingly, in the third group of 10 cases of silent PTCL, 5 cases were found to have a TCR gene configuration identical to that in the TCRαβ PTCL, as demonstrated by biallelic δ gene deletion. These 5 cases were CD3 positive. The 5 remaining cases showed a monoallelic δ gene rearrangement with a monoallelic germ line configuration in 4 and a monoallelic deletion in 1. Four of these cases were CD3 negative, which was consistent with an immature genotype the TCR commitent of which could not be ascertained.

Finally, TCRγδ PTCL consisted of a distinct clinical morphological and molecular entity whereas TCRαβ and silent PTCL had a similar presentation. (*J. Clin. Invest.* 1991. 87:666–672.) Key words: human T cell • differentiation • V genes

Introduction

The T cell antigen receptor (TCR)[1] is the antigen specific surface molecule characteristic of T cells. Two types of TCR, designated αβ and γδ, have been described (1–10). Both are heterodimeric molecules expressed in association with the CD3 protein complex (1, 10). The majority of T cells bear TCR αβ which is responsible for MHC-restricted antigen recognition (1). These T cells include two mutually exclusive subsets expressing either a $CD4^+$ $CD8^-$ or a $CD4^-$ $CD8^+$ phenotype. A minority of T cells bear TCR γδ whose recognition specificity is still under investigation (11). Most γδ T cells express the $CD4^-$ $CD8^-$ phenotype (12). The TCR δ locus, which has been recently characterized (13–22), is located within the TCR α locus on chromosome 14 and is flanked by Vα segments on its 5′ side and by Jα segments on its 3′ side. To date, one Cδ, three Jδ, three Dδ, and six Vδ segments have been reported (13–27). As a model for lymphoid differentiation, leukaemias and lymphomas in humans have provided much information for the elucidation of the developmental hierarchy among TCR genes. The TCR δ gene undergoes rearrangements and/or deletions (28–40) which might constitute the earliest recombinative event in T cell differentiation (30, 32).

In contrast to numerous studies of the TCR δ gene configuration in T cell lymphoblastic malignancies (28–36, 38, 40), few cases of nonlymphoblastic T cell lymphomas have been investigated (37, 40, 41). In this study, using five different probes, we have examined the organization of the TCR δ gene in a large series of peripheral T cell lymphomas (PTCL). The phenotype of these cases, which has been previously reported (42), allowed us to categorize them into three groups expressing either TCR αβ, TCR γδ, or neither. A correlation between the TCR δ gene status and each TCR group has been established.

Methods

Tissue samples. Frozen tissue samples from 37 cases diagnosed as peripheral T cell lymphomas on the basis of both histologic and immunologic features (excluding lymphoblastic lymphomas and mycosis fungoides) were selected for the genotypic study. In the 37 cases, the histologic and phenotypic data as well as the pattern of immunoglobulin and TCR-β gene rearrangements have been previously reported (42). Based on the reactivity of lymphoma cells with anti-CD3, βF_1, and TCRδ-1 monoclonal antibodies, the patients were categorized into three groups: $CD3^+$, βF_1^+, TCRδ-1^- (22 cases); $CD3^+$, βF_1^+, TCRδ-1^+ (5 cases); and $CD3^\pm$, βF_1^-, TCRδ-1^- (10 cases), referred to as TCR αβ, TCR γδ, and TCR silent PTCL, respectively.

DNA analysis. DNA extraction was performed as previously reported (43). 10 μg of high molecular weight DNA was digested with each appropriate restriction enzyme, fractionated in 0.8% agarose gel and blotted onto a nitrocellulose filter. Filters were P^{32} hybridized with appropriate P^{32}-labeled probes. After hybridization the filters were washed for 30 min with 2× SSC at room temperature, twice for 30 min with 0.5× SSC/0.5% SDS at 56°C and finally for 5 min with 0.5× SSC at room temperature. Autoradiography was carried out for 2–5 d at −80°C.

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1. *Abbreviations used in this paper:* D, G, R, deletion, germline, rearrangement configuration; PTCL, peripheral T cell lymphoma; TCR, T cell antigen receptor.

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DNA probes. Immunoglobulin and TCR-β genes were studied with JH and C_β probes, as previously reported (43).

The TCR-δ gene restriction map is shown in Fig. 1. TCR-δ gene rearrangements were analyzed after Bam HI and Hind III digestion using the following probes: (*a*) JδS16, specific for the $J\delta_1$ region (15); (*b*) R21XH, specific for the $J\delta_2$ region (16); (*c*) TCR $V\delta_1$, a 240-bp Eco RI-Sac I fragment isolated from the 0-240/38 cDNA clone (13) and specific for the Vδ1 region; (*d*) pDV2 SPO.S, specific for the $V\delta_2$ region (44); and (*e*) R1IEE, specific for the Cδ region (15).

In the TCR $\gamma\delta$ and TCR silent PTCL, TCR-γ gene rearrangements were analyzed by using the Jγ probe pH 60 (45) in Bam HI, Hind III, and Eco RI digests.

Rearrangements and deletions of the TCRδ gene were defined in this study according to previously reported criteria (30). When the germ line band had the same intensity as the control with no additional band, both alleles were considered in germ line configuration (GG); an additional band showed a rearranged allele (RG). When the germ line band was not visible, the δ segment was deleted either on both alleles (DD) or on one allele (RD), depending upon the presence or absence of an additional band. When the germ line band showed a decreased intensity compared with the control, the δ segment was either in a DG or RG configuration depending upon the absence or presence of an additional band. Finally, both alleles could have rearranged (RR).

Results

TCR $\alpha\beta$ PTCL. 22 cases expressed the TCR$\alpha\beta$ and most were ascribed to the diffuse mixed or diffuse large cell histologic subtype (Table I). Patient median age was 48 yr and male/female ratio was 10:1. 20 patients had stage IV disease according to the Ann Arbor classification. Lymph nodes were involved in 17 cases, bone marrow in 11 cases, skin in 8 cases, and liver in 7 cases. The spleen was enlarged in 7 cases. Treatment and survival are shown in Table I.

In 14 cases, biallelic deletions of all TCRδ sequences were detected using the $J\delta_1$ and Cδ probes (DD pattern) (Fig. 2). With these probes, monoallelic TCRδ deletions were observed in seven additional cases (DG pattern) (Fig. 2). The deletions involved the entire TCR δ gene in all cases except 4, where the Vδ1 remained in the germ line configuration. Hybridization with the Vδ2 probe was not done is this group. In case 7, a monoallelic Jδ1 rearrangement (for the sake of simplicity, the δ gene VDJ rearrangements will be referred to as VJ rearrangements) with deletion of the germ line band was found (RD pattern). The joining event involved the Vδ1 segment as demonstrated by the presence of a 12-kb band in Hind III digests using both the Jδ1 and Vδ1 probes (13).

The TCR γ gene was not studied in this group and the immunoglobulin JH and TCR β gene configurations are shown in Table I.

TCR $\gamma\delta$ PTCL. 5 cases expressed the TCR$\gamma\delta$. All had an unusual clinical presentation. Case 27 presented as a lethal midline granuloma with histologic findings consistent with a polymorphic reticulosis evolving into an overt lymphoma as previously reported (46). The 4 other cases were hepatosplenic T cell lymphoma, a new entity among PTCL with clinical histological and phenotypic features which we recently reported in 2 of them (47). The patients (3 males and 1 female) had a median age of 35 yr and presented with hepatosplenomegaly. The liver, bone marrow, and spleen had a sinusal/sinusoidal infiltration by monomorphic medium sized cells. The lymph nodes were not involved. Treatment and survival are shown in Table I.

In all 5 cases, biallelic rearrangements (RR pattern) were detected with the Jδ1 probe in Bam HI and Hind III digests (Fig. 3). In cases 23, 24, and 26, a Vδ1 Jδ1 rearrangement was observed using the Vδ1 and Jδ1 probes (Fig. 3). The other allele of these cases as well as the two alleles of cases 25 and 27, displayed a Vδ1 segment in germ line configuration whereas the Vδ2 segment was deleted as shown by hybridization with the Vδ2 probe. Therefore, the Jδ1 rearrangement did not involve either the Vδ1 or the Vδ2 segment; neither was the Vδ3 segment involved in these Jδ1 rearrangements since the Cδ probe (which detects Vδ3 rearrangement [48]) revealed only germ line bands in Bam HI digests.

The TCR γ gene had rearranged in the five cases (Fig. 3), while the immunoglobulin JH gene was in germ line configuration and the TCR β gene had rearranged in 2 out of 5 cases (Table II).

TCR silent PTCL. 10 cases expressed neither the $\alpha\beta$ nor $\gamma\delta$TCR and most were ascribed to the diffuse large cell histologic subtype (Table I). Patient median age was 50 yr and male/female ratio was 2.3:1. All patients had stage IV disease with involvement of lymph nodes in 9 cases, bone marrow in 5 cases, skin in 8 cases, and liver in 4 cases. The spleen was enlarged in 4 cases. Treatment and survival are shown in Table I.

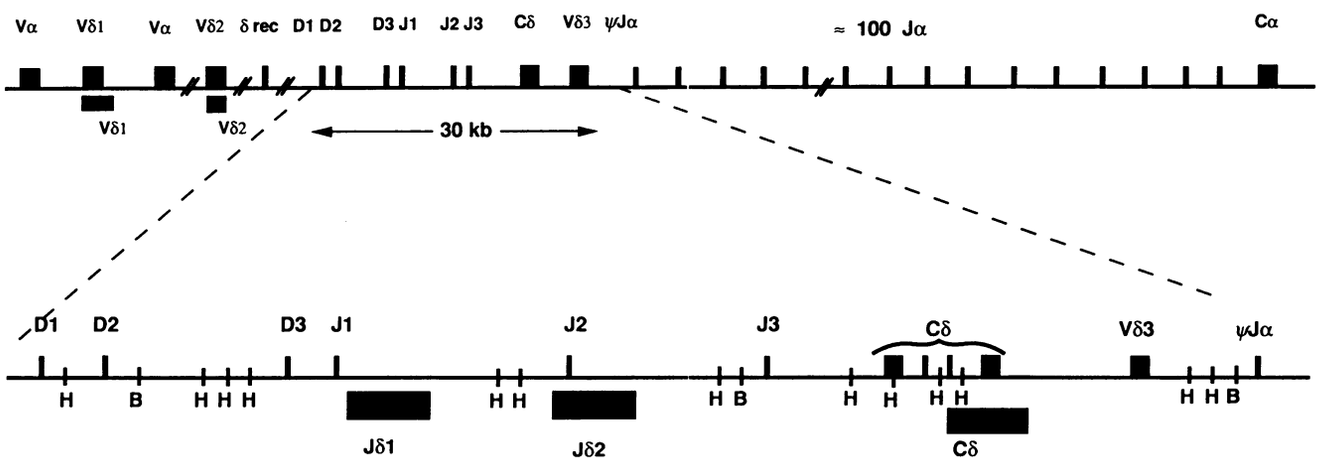

Figure 1. Genomic organization of the human TCR-δ gene. The locations of the relevant Hind III (*H*) and Bam HI (*B*) restriction enzyme sites are indicated. The solid bars below the δ gene represent the five probes which were used for hybridization of the Southern blot filters.

Table I. Histology, Phenotype, and Prognosis in 37 Cases of PTCL

Patient No.	Histology*	TCR‡	Treatment§	Survival‖	Status
1	DM	αβ	CHOP	8	dead
2	IBL	αβ	ACVBP	2	dead
3	DM	αβ	CVP	2	dead
4	DM	αβ	CHOP	15	dead
5	DM	αβ	ACVBP	35	alive
6	Uncl	αβ	ACVBP	14	dead
7	DM	αβ	CHOP	48	alive
8	ALC	αβ	ACVBP	4	dead
9	DM	αβ	ACVBP	27	dead
10	SL	αβ	CHOP	51	alive
11	DM	αβ	CVP	48	alive
12	DM	αβ	CHOP	49	alive
13	DLC	αβ	CVP	53	alive
14	DLC	αβ	ACVBP	18	dead
15	ALC	αβ	mBACOD	36	alive
16	DM	αβ	CHOP	2	dead
17	DLC	αβ	CHOP	24	alive
18	DLC	αβ	CHOP	follow up not available	
19	DLC	αβ	CHOP	2	dead
20	IBL	αβ	mBACOD	5	dead
21	DLC	αβ	CVP	follow up not available	
22	DLC	αβ	CHOP	36	alive
23	Uncl	γδ	ACVBP	25	dead
24	Uncl	γδ	ACVBP	44	dead
25	Uncl	γδ	CHOP	36	dead
26	Uncl	γδ	ACVBP	14	alive
27	DM	γδ	CHOP	2	dead
28	DLC	silent	CHOP	6	dead
29	DLC	silent	ACVBP	9	dead
30	DM	silent	CHOP	15	dead
31	DLC	silent	CHOP	6	dead
32	SL	silent	CVP	30	dead
33	DM	silent	ACVBP	36	alive
34	DLC	silent	ACVBP	7	dead
35	DLC	silent	CVP	2	dead
36	ALC	silent	CHOP	1	dead
37	ALC	silent	CHOP	22	dead

* Diffuse mixed (*DM*); immunoblastic (*IBL*); unclassifiable (*Uncl*); anaplastic large cell (*ALC*); small lymphocytic (*SL*); and diffuse large cell (*DLC*).

‡ TCR expression as determined on tissue section by βF1 and TCRδ-1 monoclonal antibody reactivity using the alkaline antiphosphatase alkaline procedure.

§ *CHOP:* cyclophosphamide, adriamycin, oncovin, prednisone, *ACVBP:* adriamycin, cyclophosphamide, velbe, bleomycine, prednisone. *CVP:* cyclophosphamide, VM26, prednisone. *mBACOD:* methotrexate, bleomycin, cyclophosphamide, oncovin, dexamethasone.

‖ Months.

In 5 cases, TCRδ biallelic deletion was demonstrated by using the Vδ1, Jδ1, Jδ2, and Cδ probes (DD pattern) (Fig. 4).

In the 5 other cases, a monoallelic rearrangement (RD or RG pattern) involving either the Jδ1 (cases 29, 35, 37) or the Jδ2 segment (cases 34, 36) was detected with the Jδ1 and Jδ2 probes (Fig. 4). When the Jδ1 segment was rearranged, the Jδ2 segment was in germ line configuration (Fig. 4). When the Jδ2 segment was rearranged, the Jδ1 segment was deleted. Using the Vδ1 probe in Hind III digests, the Vδ1 segment was shown to be rearranged in a Vδ1Jδ1 joining in 3 cases with a 12-kb band and in a Vδ1 Jδ2 joining in 2 cases with a 7.3-kb band (Fig. 4). In all these 5 cases, the Cδ segment was in germ line configuration as shown by the Cδ probe.

All 10 cases had rearranged the TCRγ locus as detected by the Jγ1 probe, while the JH gene was in germline configuration in all cases and the Cβ gene had rearranged in 8 out of 10 cases (Table III).

Discussion

We have analyzed the recombinative events involving the TCR δ locus in 37 cases of PTCL. These were categorized into 3 phenotypic groups according to TCR expression. 22 cases expressed TCR αβ, 5 cases γδ, and 10 cases neither αβ nor γδ. Using 5 probes specific for the Jδ1, Jδ2, Vδ1, Vδ2, and Cδ segments, Southern blot analyses demonstrated deletions and/or rearrangements in all 37 cases of PTCL.

In all cases of TCR αβ PTCL either biallelic (DD) or monoallelic (DG, RD) deletions were observed. 1 case exhibited a monoallelic Vδ1 Jδ1 rearrangement in addition to the deleted allele. As expected, the TCR αβ expression implied the deletion of the TCR δ gene locus associated with the TCR α gene rearrangement. The TCR δ locus, nested between the Vα and Jα regions, is deleted in Vα Jα joining (23), occasionally sparing the Vδ1 segment which thus remains in germ line configuration (48) as found here in 4 cases. The TCR δ gene configuration in TCR αβ PTCL was in keeping with the findings of others in T ALL (33, 35) and T cell clones (48, 49) expressing the TCR αβ. During differentiation, whether the TCR δ and α genes rearrange successively in a single pathways or in two different pathway remains controversial (50–52). In our series of β PTCL, most alleles, i.e., 36/44, were deleted, which precludes any conclusion regarding the status of the δ gene before α gene rearrangement. Thus, the deletion could involve a δ gene either in germ line configuration or when rearranged.

The 5 cases of TCR γδ PTCL had a biallelic TCR δ rearrangement (RR). All 5 cases had a peculiar clinical presentation clearly distinct from TCRαβ and silent PTCL. 1 case was a midline granuloma evolving into a PTCL (46) and 4 cases were hepatosplenic PTCL, 2 of which we recently reported as a new entity (47). The tropism of malignant γδ T cells for the sinuses of spleen, bone marrow, and the sinusoids of the liver is remarkable with regard to the preferential localization in the splenic red pulp of their normal counterpart (42). In this report we have extended the study of the genotypic configuration of TCR genes to 5 cases of TCR γδ PTCL and the V and J segments involved in the TCRδ rearrangement have been characterized. 3 of the 5 cases displayed a productive Vδ1 Jδ1 rearrangement which has been reported as the most frequent pattern in T ALL and T lymphoblastic lymphomas expressing the TCR γδ (33–35). The δTCS1$^+$/anti-Vδ2$^-$ phenotype of the lymphoma cells (47) was in agreement with the genotypic findings and has been reported as that of the major subset of γδ T cells in normal thymus and spleen (54). The TCRδ productive rearrangements in the other 2 cases also involved the Jδ1 segment. However,

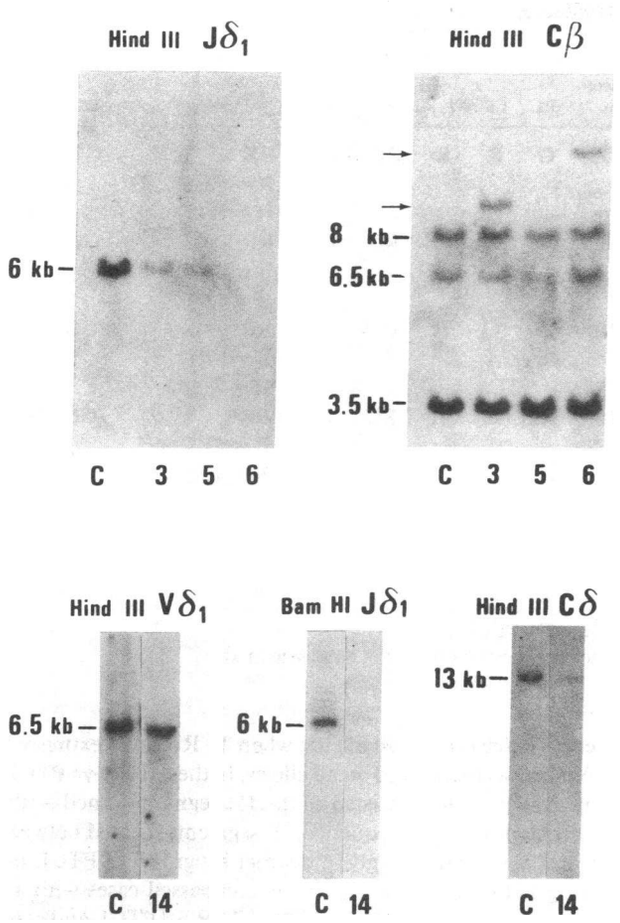

Figure 2. Representative TCRδ gene deletions in TCR αβ PTCL. Patient number is given below each lane. *C*, control DNA from granulocytes. Germ line bands are indicated by thin lines near the lane *C* and their sizes are given in kb. Rearranged bands are shown by arrows. On the top figure, two DG pattern and one DD pattern are shown after Jδ1 hybridization. The same filter was rehybridized with the Cβ probe in order to demonstrate that an equal amount of DNA was loaded in each lane. The bottom figure illustrates case No. 14 with one allele having deleted Jδ1 and Cδ segments while the Vδ1 segment has remained in germ line configuration.

the V segments that joined Jδ1 were neither Vδ1, Vδ2, nor Vδ3 as demonstrated with the appropriate δ probes. In addition, the δ chain encoded by these VJδ1 combinations was recognized by the TCR-δ1 monoclonal antibody, which reacts with all δ chains, and not by the δ TCS-1 and anti-Vδ2 antibodies which react with an epitope encoded by the Vδ1Jδ1 pairing and the Vδ2 segment, respectively (see reference 47). In these 5 cases of γδ PTCL, the 5 rearranged and nonproductive alleles also involved the V segments located between Vδ1 and Vδ2. Indeed, the Vδ1 segment was in germ line configuration and the Vδ2 segment was deleted, showing that Jδ1 rearranged with V segments located 3′ to Vδ1 and 5′ to Vδ2. These V segments might belong to the Vα pool as reported previously in a γδ T cell clone (55). Alternatively, other Vδ segments embedded in the Vα family might rearrange with Jδ1 as suggested in δTCS1$^-$ clones derived from the thymus (56). Taken together, the TCR γδ PTCL had a genotype and phenotype in marked contrast to those of T cell γδ clones derived from the peripheral blood of normal individuals which use preferentially a Vδ2 Jδ1 joining (54, 57–59).

The third group of 10 patients consisted of TCR silent PTCL (Table IV). Their clinical and morphological characteristics were similar to those of TCRαβ PTCL. 5 of these displayed a TCR genotype identical to that in the group of TCR αβ PTCL, namely a biallelic TCRδ deletion with a TCRβ rearrangement. In addition, the deletion of Vδ1 embedded in the Vα pool indicated a Vα-Jα rearrangement. Therefore, the lymphoma cells seemed to be committed to TCRαβ expression. In addition, they were CD3$^+$ on tissue sections. The βF1$^-$ status could be explained in two ways: either the level of β chain expression was below the detection threshold of the immunostaining or nonproductive rearrangements or a translational dysregulation prevented the β chain synthesis. Northern blot analyses were not performed because of insufficient material. The possibility that some of these 5 cases arose from cells initially expressing TCRαβ cannot be excluded, since a pheno-

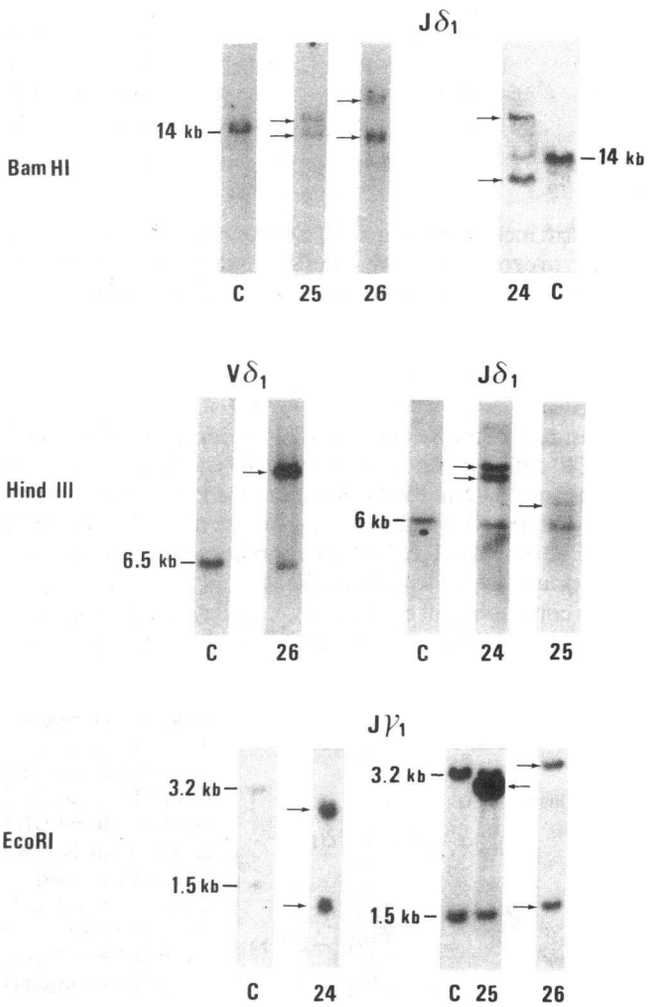

Figure 3. Representative TCR δ and γ gene rearrangements in TCR γδ PTCL. Patient number, control DNA, germ line bands, and rearrangements are indicated as in Fig. 1. Note the additional 12-kb band in Hind III digests, which shows a Vδ1 Jδ1 rearrangement, as demonstrated here by the Vδ1 probe in case 26 and by the Jδ1 probe in case 24.

Table II. Genotype of the TCR αβ PTCL

Patient No.	JH	Cβ	Vδ$_1$	Jδ$_1$	Cδ
1	G	R	DD	DD	DD
2	G	R	DD	DD	DD
3	G	R	DG	DG	DG
4	G	R	DD	DD	DD
5	G	G	DG	DG	DG
6	G	R	DD	DD	DD
7	G	R	RD	RD	DG
8	G	R	DD	DD	DD
9	G	R	GG	DG	DG
10	G	R	DG	DG	DG
11	G	R	DD	DD	DD
12	G	R	DD	DD	DD
13	G	R	DD	DD	DD
14	R	R	GG	DG	DG
15	G	G	GG	DG	DG
16	G	R	GG	DG	DG
17	G	R	DD	DD	DD
18	G	R	DD	DD	DD
19	G	R	DD	DD	DD
20	G	R	DD	DD	DD
21	G	R	DD	DD	DD
22	G	R	DD	DD	DD

typic drift including the loss of TCR expression can occur along tumor progression while the TCR genotype remains unchanged (47). 5 additional cases of TCR silent PTCL had a monoallelic δ gene rearrangement. 3 of them consisted of a Vδ1 Jδ1 joining and the other 2 of a Vδ1 Jδ2 joining. In these 5 cases unrearranged alleles were either germ line in 4 cases (RG), or deleted in 1 case (RD). 4 cases (1 RD + 3 RG) were CD3$^-$ suggesting that the lymphoma cells were immature with respect to TCR differentiation. Their genotype was in contrast to the unequivocal DD and RR genotype of the PTCL expressing the CD3 antigen. Alternatively, the genotype of TCR silent PTCL could be that of a T cell which normally would have died but was rescued by the leukaemic events.

In conclusion, all cases of PTCL displayed a recombinative event in the TCRδ locus. When TCR αβ was expressed, the δ gene was deleted on most alleles; when TCR γδ was expressed, the δ gene was rearranged on all alleles. In these TCR γδ PTCL, the rearrangements consisted of the Jδ1 segment joined to the Vδ1 segment or yet unsequenced V segments located between Vδ1 and Vδ2. Interestingly, a distinct subgroup of PTCL expressing neither TCR αβ nor γδ encompassed cases with a δ gene configuration identical to that in TCR αβ PTCL and cases with an "immature" genotype which differed from that in both

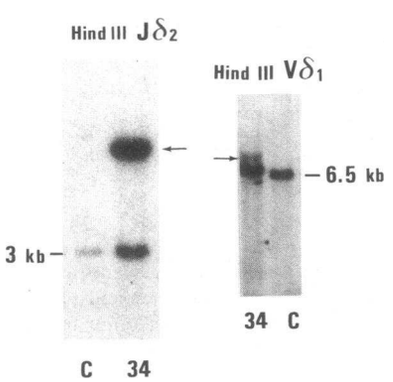

Figure 4. Representative TCRδ gene rearrangement in TCR silent PTCL. Patient number, control DNA, germ line bands, and rearrangements are indicated as in Fig. 1. The patient 34 illustrated here had a Vδ1 Jδ2 rearrangement demonstrated by an additional 7.3-kb band in Hind III digests which were hybridized with the Vδ1 and Jδ2 probes.

Table III. Genotype of the TCR γδ PTCL

Patient No.	JH	Cβ	Jγ$_1$	Vδ$_1$	Vδ$_2$	Jδ$_1$	Jδ$_2$	Cδ
23	G	R	R	RG	DD	RR B = >23/10, 5* H = 12/5	GG	GG
24	G	G	R	RG	DD	RR B = >23/12 H = 12/10	GG	GG
25	G	G	R	GG	DD	RR B = 15/12, 5 H = 7, 6/G	GG	GG
26	G	G	R	RG	DD	RR B = >23/12, 5 H = 12/5	GG	GG
27	G	R	R	GG	DD	RR B = 15/12, 5 H = 7, 6/G	GG	GG

B, Bam H1; *H*, Hind III.
* Numbers correspond to the band size in kb.

Table IV. Genotype of the TCR silent PTCL

Patient No.	JH	Cβ	Jγ$_1$	Vδ$_1$	Jδ$_1$	Jδ$_2$	Cδ
28	G	R	R	DD	DD	DD	DD
29	G	G	R	RG	RG B = >23 H = 12	GG	GG
30	G	R	R	DD	DD	DD	DD
31	G	R	R	DD	DD	DD	DD
32	G	R	R	DD	DD	DD	DD
33	G	R	R	DD	DD	DD	DD
34	G	G	R	RG	DG	RG B = >23 H = 7, 3	GG
35	G	R	R	RG	RG B = >23 H = 12	GG	GG
36	G	R	R	RG	DD	RD B = >23 H = 7, 3	GD
37	G	R	R	RG	RG B = >23 H = 12	GG	GG

* Patients 28–33: CD3$^+$; patients 34–37: CD3$^-$.

the TCR $\alpha\beta$ and TCR $\gamma\delta$ PTCL. Most importantly, TCR$\gamma\delta$ PTCL consisted of a clearly defined clinical, morphological, and molecular entity whereas TCR$\alpha\beta$ and silent PTCL had a similar presentation.

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