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Relationships among expression, transcription and rearrangement of T-cell receptor β gene in T-cell lymphomas

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Summary. The expression of T-cell receptors (TCR) in malignant lymphomas was examined immunohistochemically by monoclonal antibodies which react with the TCRβ or TCRδ chain. TCRβ was expressed in 16 out of 47 non-Hodgkin's lymphomas. These included 15 T-cell lymphomas and 1 Ki-1 lymphoma. The anti-TCRβ chain antibody, βF1, did not react with 26 B-cell lymphomas, 1 Ki-1 lymphoma or 6 Hodgkin's disease. The anti-TCRδ chain antibody, TCRδ1, did not react with any type of malignant lymphoma. Although TCRβ and CD3 were co-expressed in normal lymphoid tissues and most T-cell lymphomas, 3 cases of CD3+CD4+CD8− T-cell lymphoma failed to express TCRβ. TCRβ and Ig J_H gene configurations in malignant lymphomas were examined by Southern hybridization. Although each of 9 T-cell lymphomas had a rearranged TCRβ locus, TCRβ gene rearrangement in the 3 cases of βF1−CD3+ T-cell lymphomas was demonstrated by Southern blot. No transcripts of the TCRβ gene could be found in 2 out of the 3 βF1−CD3+ T-cell lymphomas by Northern blot, indicating the lack of TCRβ protein expression to be due to non-transcription of the TCR gene. Loss of TCRβ proteins in these T-cell lymphomas is thus quite likely to be associated with T-cell tumour activation and progression, since 3 βF1−CD3+ T-cell lymphomas expressed CD25 (interleukin-2 receptor) to a high degree.

Key words: T-cell receptors – Malignant lymphomas – Immunohistochemistry – Transcription – Rearrangement

Introduction

Recent research has clarified the molecular structure and functional properties of the T-cell receptor (TCR) (Yanagi et al. 1984; Minden and Mak 1986). The TCR is a heterodimer consisting of two disulphide-linked variable glycoproteins with immunoglobulin-like organization, and is thought to bind both the antigen and molecules encoded by the major histocompatibility complex (Minden and Mak 1988). There are two types of TCR, one consisting of the α-β heterodimer (TCRαβ) and another containing γ and δ chains (TCRγδ). Most peripheral T-cells express TCRαβ, and a small sub-population of T-cells possesses TCRγδ (Chan et al. 1988; Groh et al. 1989). The clonality and cell lineage of T-cells for various lymphoproliferative disorders have been indicated by gene rearrangement studies by using molecular probes of TCR genes, since surface immunophenotypes in T-cell tumours were found inadequate for this purpose (Cossman et al. 1988; Griesser et al. 1989). Recently, immunohistochemical examination of TCRβ protein in lymphoid tissues and malignancies has been carried out using anti-TCRβ monoclonal antibodies (mAbs) (Chan et al. 1988; Ng et al. 1988). Although TCR are associated with CD3 molecules in T-cells which are essential for signal transduction during T-cell activation, loss of the TCRβ-CD3 complex and lack of either TCRβ or CD3 were observed in T-cell lymphomas (Chan et al. 1988; Ng et al. 1988). However, the molecular basis for such aberrant expression of TCRβ in fresh lymphoma cases has not been reported. In the present study, TCR chain expression and TCRβ gene rearrangements in lymphoid malignancies were examined using anti-TCR mAbs and TCRβ gene probe, and the molecular basis of TCRβ expression is discussed.

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Materials and methods

Lymphoid tissues from 47 cases of non-Hodgkin's lymphomas, 6 of Hodgkin's diseases and 9 normal tissues were obtained from the Kitasato University Hospital. Five normal lymph nodes and 2 tonsils were taken from specimens removed surgically for diagnostic or therapeutic purposes. Two fetal thymuses were obtained at the time of autopsy. Lymph nodes of 7 non-Hodgkin's lymphomas listed in Table 1 (cases 1, 10, 14 and 16) and Table 2 (3 of diffuse centroblastic/centrocytic type) were obtained at the time

Table 1. Expression of TCRβ and TCRδ in T-cell and Ki-1 lymphomas

Case no.	Histology		Surface markers						
			TCRβ	TCRδ	CD3	CD4	CD8	CD25	CD30
1	Pleom[a] medium-large[b]	High[c]	++	−	+++	++	−	+	−
2	Angioimmunoblastic	Low[d]	+++	−	+++	++	−	++	−
3	Pleom medium	High	+++	−	+++	++	−	+	−
4	Angioimmunoblastic	Low	++	−	+++	+++	−	+	−
5	Angioimmunoblastic	Low	+++	−	+++	+++	−	+	−
6	Angioimmunoblastic	Low	++	−	+++	+++	−	+	−
7	Angioimmunoblastic	Low	++	−	+++	++	−	+	−
8	Pleom medium	High	+++	−	+++	+++	−	−	−
9	Pleom medium-large	High	++	−	+++	++	−	+	−
10	Pleom medium-large	High	++	−	+++	+++	−	+++	−
11	Pleom medium-large	High	+++	−	+++	++	−	+	−
12	Pleom large	High	++	−	+++	−	+++	++	+
13	Pleom large	High	−	−	+++	++	−	++	−
14	Pleom large	High	−	−	++	++	−	+++	−
15	Pleom large[e]	High	−	−	+++	+++	−	+++	−
16	Pleom large[e]	High	++	−	+++	+++	−	++	−
17	Pleom medium-large	High	++	−	+++	+++	−	+	−
18	Angioimmunoblastic	Low	++	−	+++	−	++	++	+
19	Lymphoblastic[f]	High	−	−	−	−	−	−	−
20	Large cell anaplastic	High	+	−	−	−	−	+++	+++
21	Large cell anaplastic	High	−	−	−	+	−	++	++

[a] Pleom, pleomorphic; [b] medium-large, medium and large cell; [c] high, high-grade malignancy; [d] low, low-grade malignancy; [e] HTLV-I positive; [f] CD1−3−4−7+8−

Table 2. Expression of TCR in B-cell lymphomas

Histology	No. of cases	Expression of TCR	
		TCRβ	TCRδ
Low grade			
Lymphocytic	2	0	0
Centroblastic/ centrocytic follicular	4	0	0
Centrocytic	2	0	0
Centroblastic/centrocytic diffuse	13	0	0
High grade			
Centroblastic	3	0	0
Immunoblastic	2	0	0
Total	26	0	0

of autopsy. The remaining cases of non-Hodgkin's lymphomas and 6 of Hodgkin's diseases were obtained by biopsy of lymph nodes (44) and tonsils (cases 12 and 18 listed in Table 1). Lymphoid tissues were snap-frozen in OCT compound (Miles Scientific, Naperville, Ill., USA) and stored at −80° C until use. Fresh frozen 4-μm-thick tissue sections were cut serially on a cryostat, air-dried for 30 min and post-fixed in acetone for 3 min at 4° C. This was followed by fixation in 10% formalin and processing to obtain 3-μm paraffin sections. Routine paraffin sections were examined immunohistochemically as described previously (Kasai et al. 1986). Histological diagnosis was conducted according to the updated Kiel classification (Suchi et al. 1987; Hui et al. 1988; Stansfeld et al. 1988).

For immunoperoxidase staining the sections were soaked in 0.03% hydrogen peroxide in phosphate-buffered saline (PBS) for 5 min at room temperature to eliminate endogenous peroxidase activity. After being washed in PBS, the sections were incubated with primary mAbs for 1 h at room temperature. Immunoperoxidase staining was then performed by the avidin-biotin-complex (ABC) method (Hsu et al. 1981). The reagents used for this purpose were purchased from Vector Laboratory (Burlingame, Calif., USA). Dilution and incubation time were as specified in the staining procedure manual of the Vectastain ABC Kit. The sections were coloured with 0.05 M TRIS-HCl buffer, pH 7.6, containing 20 mg/100 ml of 3,3′-diaminobenzidine tetrahydrochloride and 0.05% hydrogen peroxide for 5–10 min. The sections were washed in PBS, counterstained with haematoxylin and then mounted in plastic or glycerol in PBS. As negative controls, the sections were incubated with normal mouse serum or normal mouse IgG. Finally, they were stained by the ABC method.

The following MAbs were used in the present study: anti-TCRβ chain antibody, βF1, and anti-TCRδ chain, TCRδ1, were obtained from T-cell Sciences, Cambridge, Mass., USA (Brenner et al. 1987; Band et al. 1987). Antibodies reactive with T-cells, Leu1 (CD5), Leu2 (CD8), Leu3 (CD4), Leu4 (CD3), Leu6 (CD1), and Leu9 (CD7) were purchased from Becton-Dickinson, Mountain View, Calif., USA. B1 (CD20) was used to detect B-cells in tissue, and purchased from Coulterclone, Hialeah, Florida, USA. RSC-1 (Ki-1, CD30) and anti-interleukin-2 receptor (IL-2R, CD25) antibodies were purchased from Dakopatts, Copenhagen, Denmark. LeuM1 (CD15) was purchased from Becton-Dickinson. mAb reactive with sialosyl X (SLEX) was supplied by UCLA Tissue Typing Laboratory, Calif. (Fukushima et al. 1984). Each mAb was used at a final concentration of approximately 5 μg/ml.

Immunoreactivity was graded as follows: (−) none, (+) scattered (0–10% of antigen-positive cells in the sections), (++) prominent (10%–50%), and (+++) diffuse (>50%).

In order to perform Southern hybridization high-molecular-weight DNA was extracted from lymphoid tissues. Genomic DNA from each sample (4 μg) was digested with EcoRI or HindIII (Boehringer, Mannheim, FRG) and size-fractionated by 0.75% agarose gel electrophoresis. The DNA was then transferred from the gel to nitrocellulose filter paper by the Southern transfer technique (Southern 1975). All such filters were hybridized at 65° C

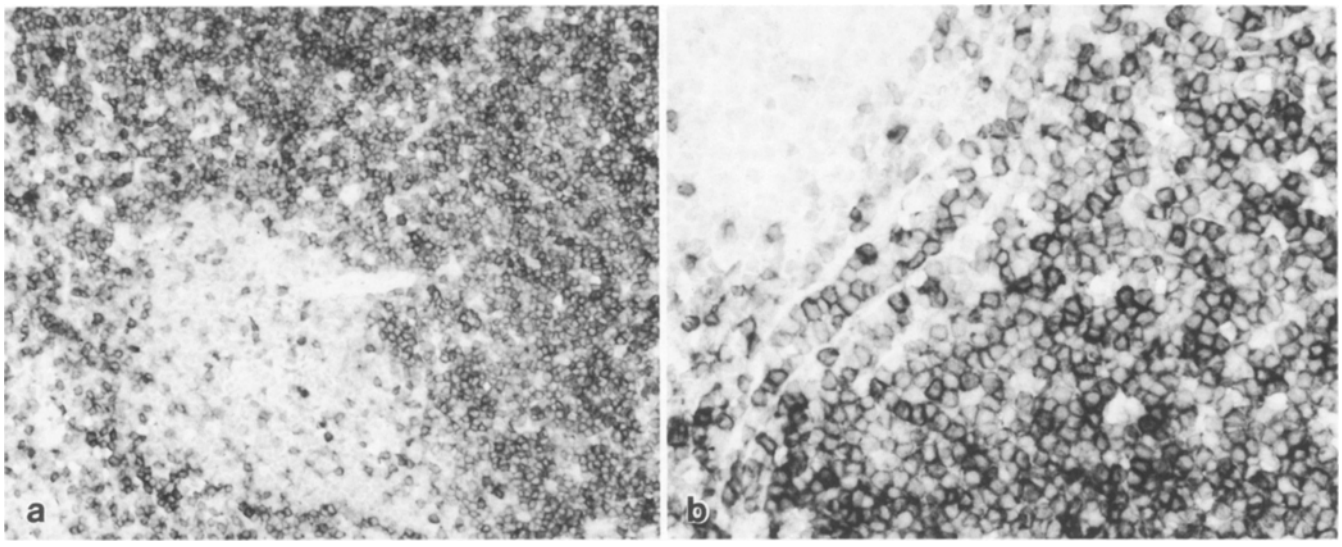

Fig. 1a, b. Immunoperoxidase staining by βF1 of sections of a lymph node. The majority of lymphocytes in paracortical areas were stained with βF1. **a** $\times 100$; **b** $\times 200$

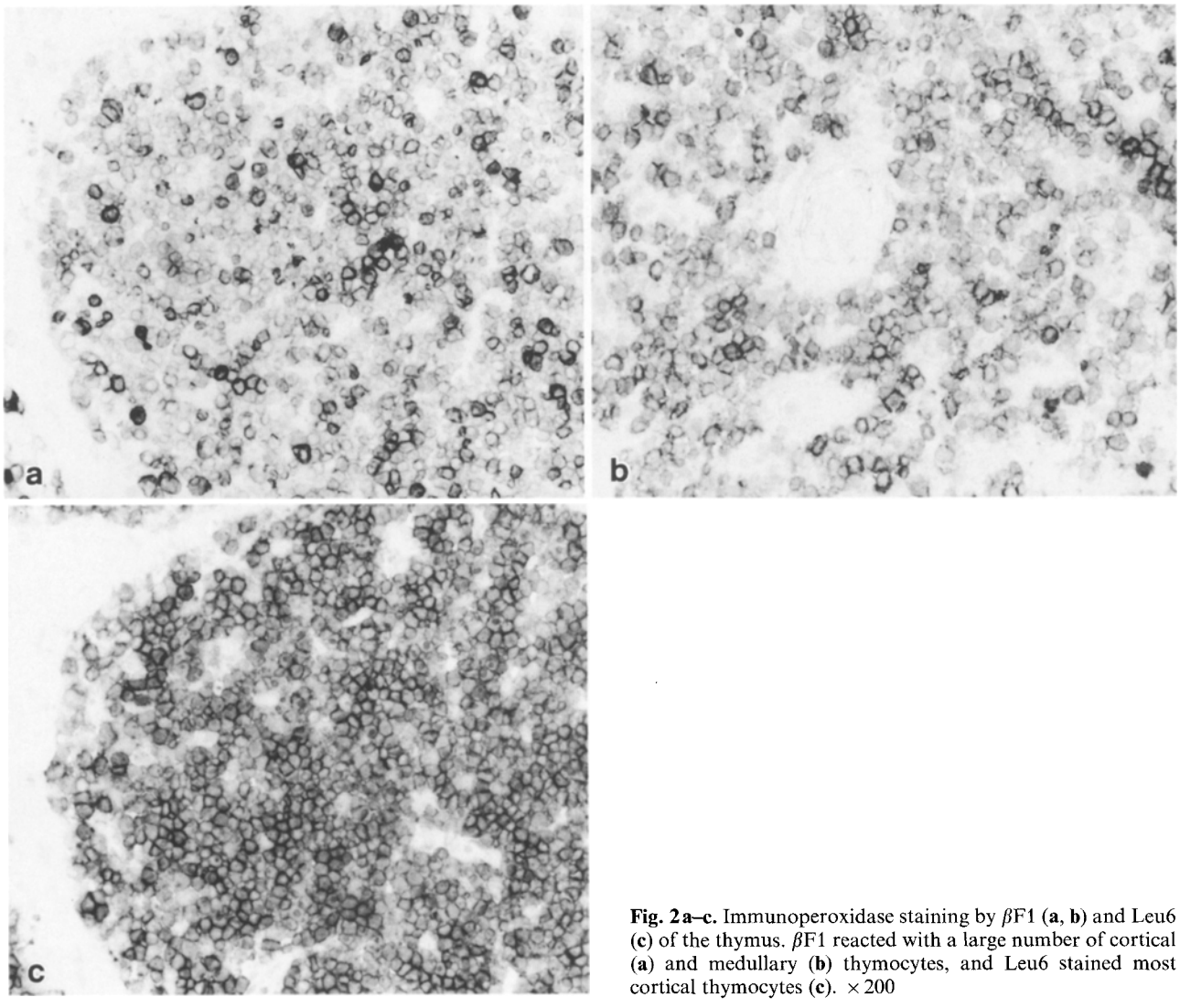

Fig. 2a–c. Immunoperoxidase staining by βF1 (**a**, **b**) and Leu6 (**c**) of the thymus. βF1 reacted with a large number of cortical (**a**) and medullary (**b**) thymocytes, and Leu6 stained most cortical thymocytes (**c**). $\times 200$

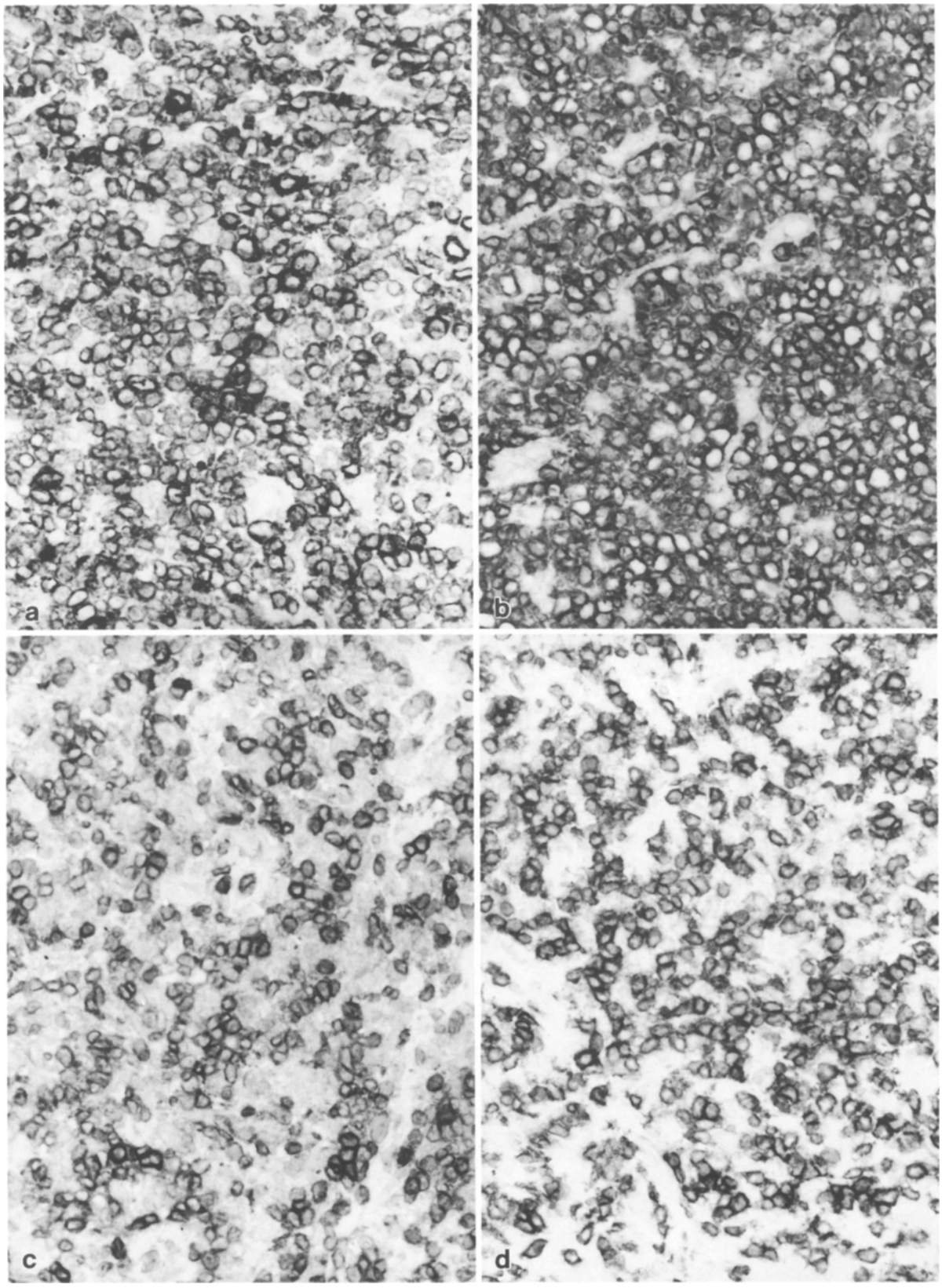

Fig. 3a–d. Immunoperoxidase staining by βF1 (**a**, **c**) and Leu4 (**b**, **d**) on sections of malignant lymphomas (case 10, **a**, **b**; case 6, **c**, **d**). βF1 and Leu4 reacted with most lymphoma cells. $\times 200$

for 24–48 h with DNA probes labelled with ^{32}P (Amersham, Little Chalsont, Buckinghamshire, England) by the random primed DNA labelling method (Feinberg and Vogelstein 1983). Following hybridization, the filters were washed for 1 h in 0.3 × standard saline citrate (SSC: 0.15 M NaCl, 0.015 M trisodium citrate), and 0.1% sodium dodecyl sulphate (SDS) solutions followed by autoradiography at −70° C.

The immunoglobulin (Ig) gene probe used in this study was a 6 kb BamHI-HindIII genomic fragment containing the J region of the Ig heavy-chain gene (J_H) [provided by Dr. P. Leder, Harvard Medical School, Boston (Ravetch et al. 1981)]. The TCR gene probe comprised 312 base pairs of BglII-StuI fragment of the DNA clone, YT35, having the constant region of the TCR β-chain gene (provided by Dr. T. Mak, University of Toronto, Toronto) (Yanagi et al. 1984). Figure 4 shows the structural relationships of TCRβ and Ig J_H gene probes to their respective loci. The criteria for the rearrangements of TCRβ and Ig J_H genes was the appearance of new bands on the autoradiographs of lymphoma DNA plus partial or complete deletion of germ-line bands.

For Northern hybridization, extraction of total cellular RNA was performed as described previously (Maniatis et al. 1982) according to the guanidinium/hot phenol method. Polyadenylated RNA was selected by oligo (dT)-cellulose chromatography. Northern transfer and filter hybridization were conducted by the method of Thomas (1980). Hybridization was performed at 42° C in 50% (v/v) formamide/5 × SSC/50 mM of sodium phosphate buffer (pH 6.8)/0.1% bovine serum albumin/0.1% Ficoll 400/0.1% polyvinyl pyrrolidone/5% dextran sulphate containing sonicated *Escherichia coli* DNA at 100 μg/ml and yeast RNA at 100 μg/ml. After hybridization, the filter was washed five times in 0.1 × SSC + 0.1% SDS at 65° C.

Results

The expression of TCRβ and TCRδ in lymphoid tissues such as lymph node, tonsil, and thymus was examined immunohistochemically. Immunoreactivity of the anti-TCRβ mAb, βF1, was detected in most lymphocytes of paracortical or interfollicular areas in lymph nodes (Fig. 1a, b) and tonsils. βF1 immunoreactivity was evident in a few lymphocytes of the germinal centre in lymph nodes and tonsils. The pattern of its reactivity was similar to that of Leu4 (CD3) in these lymphoid tissues. Although βF1 reacted with a large number of cells in both the cortex and medulla of the thymus, the number of thymocytes expressing TCRβ were less than those expressing CD1, CD4 and CD8 (Fig. 2a–c). A small number of T-cells was found to bear TCRδ in lymph node, tonsil and thymus.

The 47 non-Hodgkin's lymphomas were classified histologically according to the updated Kiel classification (Stansfeld et al. 1988) (Tables 1 and 2). The 6 cases of Hodkin's disease were examined and classified according to Rye classification (Table 4). Non-Hodgkin's lymphomas were also classified immunologically by surface markers. Lymphomas expressing at least one of T-cell markers such as CD3, 4 and 8 were classified as T-cell lymphomas. One lymphoblastic type (case 19) had CD7 but not CD1, 3, 4, 8, or 20 (Table 1). Lymphomas expressing CD20 but not the surface markers of T-cells were considered B-cell lymphomas (Table 2). Ki-1 positive large cell anaplastic lymphomas (cases 20, 21) were positive for CD25, 30 and HLA-DR (Table 1; Stein et al. 1985).

Table 1 summarizes the expression of TCR in 19 T-cell and 2 Ki-1 lymphomas. TCRβ was expressed in 15 of 19 T-cell lymphomas which included 13 CD3+ CD4+ CD8− T-cell lymphomas (Fig. 3) and 2 CD3+ CD4− CD8+ T-cell lymphomas (Table 1). A T-cell lymphoblastic lymphoma and 3 CD3+ CD4+ CD8− T-cell lymphomas including 1 adult T-cell leukaemia (ATL) were negative for TCRβ. Although no simple correlation could be found between morphological appearance and βF1 expression in T-cell lymphomas, 3 βF1−CD3+CD4+ cases were high-grade lymphomas and expressed IL-2R (CD25) to a high degree (cases 13–15, Table 1). No TCRβ could be detected in 26 B-cell lymphomas (Table 2). Although 1 Ki-1 lymphoma was negative for TCRβ, a small number of cells in 1 Ki-1 lymphoma expressed TCR but not CD3. No TCRδ expression in T-cell, B-cell or Ki-1 lymphomas could be found (Tables 1, 2).

TCRβ and Ig J_H chain gene configurations were examined by Southern hybridization (Fig. 5, Table 3). No rearrangement of Ig J_H chain genes was noted in 9 T-cell lymphomas, 2 Ki-1 lymphomas or 3 Hodgkin's diseases (Table 3). All 9 T-cell lymphomas each showed a rearranged TCRβ locus (Table 3). Such rearrangement was not found in 2 Ki-1 lymphomas. Neither rearrangement was observed in 3 cases of Hodgkin's disease (Table 3), although 11 kb bands of EcoRI digested DNA obtained from 2 of 3 cases were diminished or deleted (data not shown), indicating there to be polyclonal populations of T-cells in the lymphomas, as previously reported (Griesser et al. 1987; Roth et al. 1988).

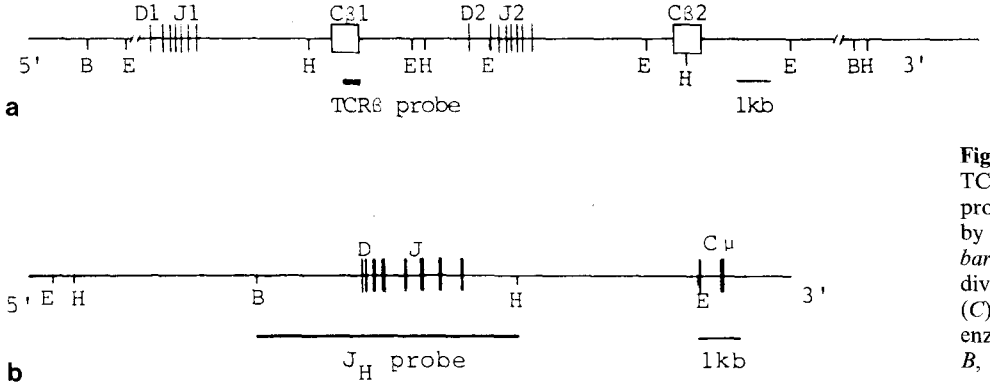

Fig. 4. Restriction maps of genomic TCRβ (*A*) and Ig J_H (*B*) genes. Each probe used in this study is indicated by a *thick bar*. *Open boxes* and *vertical bars* represent exons including the diversity (*D*), joining (*J*) and constant (*C*) DNA segments. Restriction enzyme abbreviations are as follows: *B*, Bam HI; *E*, EcoRI; *H*, Hind III

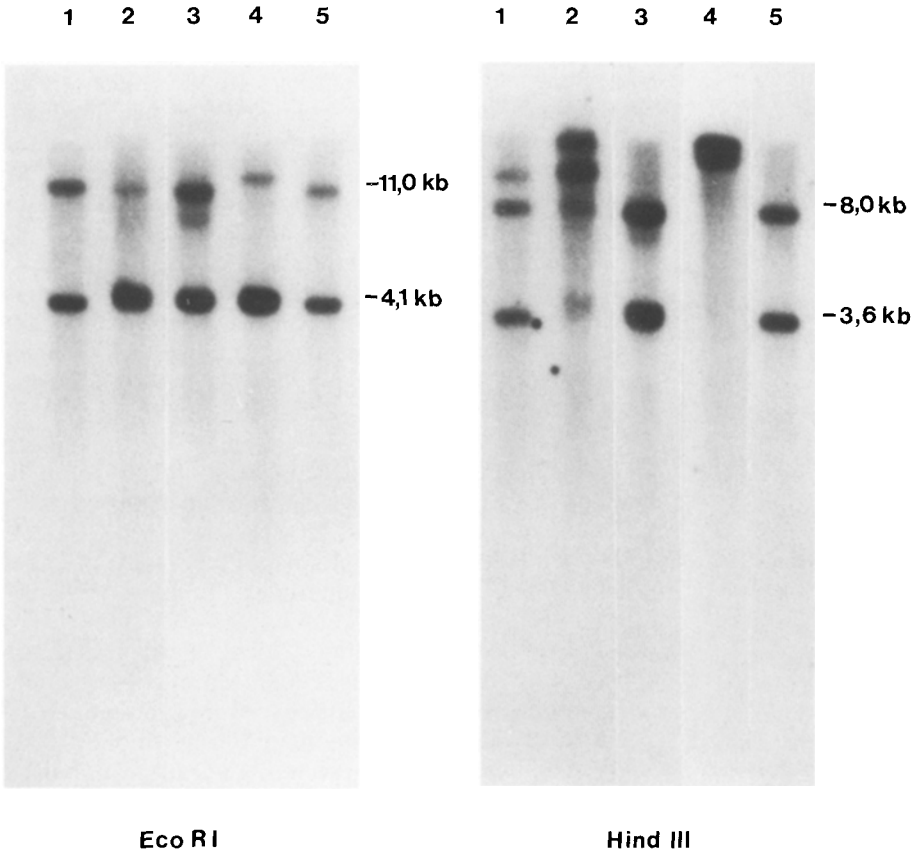

Fig. 5. The rearrangement patterns of T-cell receptor β chain genes in three patients with T-cell lymphomas. *Lanes 1–5* show the patterns following digestion with EcoRI and HindIII for 3 patients selected from Table 3 and for two controls. *Lane 1*, case 14; *lane 2*, case 15; *lane 3*, case 13; *lane 4*, MOLT-4F (T-cell leukaemic cell line); *lane 5*, germ-line control. The germ-line positions are indicated on the right in kilobases (kb). EcoRI digestion gave 2 germ-line bands of 11.0 kb and 4.1 kb. The germ-line configuration digested by HindIII gave 2 bands of 8.0 kb and 3.6 kb

Table 3. Immunophenotypes and rearrangements of immunoglobulin and T-cell receptor genes in malignant lymphomas

Case no.		Surface markers[a]						Rearrangements[b]			
		TCRβ	CD3	CD4	CD8	CD20	CD30	TCRβ		Ig J_H	
								E	H	E	H
1	T	++	+++	++	−	−	−	R	G	G	G
2	T	+++	+++	++	−	−	−	G	R	G	G
3	T	+++	+++	++	−	−	−	R	R	G	G
6	T	++	+++	+++	−	−	−	R	R	G	G
10	T	++	+++	+++	−	−	−	R	R	G	G
13	T	−	+++	++	−	−	−	R	R	G	G
14	T	−	++	++	−	−	−	G	R	G	G
15	T	−	+++	+++	−	−	−	R	R	G	G
18	T	++	+++	−	++	−	−	G	R	G	G
20	Ki-1	+	−	−	−	−	+++	G	G	G	G
21	Ki-1	−	−	+	−	−	++	G	G	G	G
48	HD	−	−	−	−	−	+	G	G	G	G
49	HD	−	−	−	−	−	++	G	G	G	G
50	HD	−	−	−	−	−	++	G	G	G	G

[a] T, T cell lymphoma; Ki-1, Ki-1 positive large cell lymphoma; HD, Hodgkin's disease
[b] E, EcoRI; H, HindIII; R, rearrangements; G, germ line configuration

With regard to the expression of TCRβ, TCRβ gene rearrangement was found not only in βF1 positive T-cell lymphomas but also in 3 T-cell lymphomas negative for βF1 (cases 13–15; Table 3, Fig. 5).

βF1 and TCRδ1 failed to react with Hodgkin's or Reed-Sternberg (RS) cells in 6 cases of Hodgkin's disease (Table 4), although small lymphocytes were stained with βF1. Both Hodgkin's and RS cells were stained with LeuM1, RSC1 and IL-2R. SLEX was expressed on the lymphocytic and histiocytic variants of RS cells of one

Table 4. Expression of TCRβ and TCRδ in Hodgkin's lymphomas

Case no.	Histological subtype	TCRβ	TCRδ	CD30	CD15	SLEX
48	Lymphocyte predominance	−	−	+	−	+
49	Nodular sclerosis	−	−	++	++	−
50	Mixed cellularity	−	−	++	++	−
51	Mixed cellularity	−	−	++	++	−
52	Mixed cellularity	−	−	++	++	−
53	Lymphocyte depletion	−	−	+	+	−

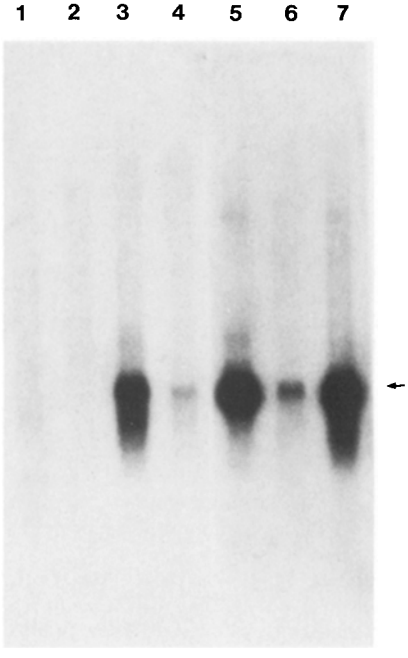

Fig. 6. Northern hybridization analysis. Poly(A)$^+$ was denatured and separated in a 1% agarose gel containing formaldehyde and hybridized with TCRβ probe. *Lane 1*, case 13 (βF1−CD3+ T-cell lymphoma listed in Table 5); *lane 2*, case 14 (βF1−CD3+ T-cell lymphoma listed in Table 5); *lane 3*, Hodgkin's lymphoma (case 50 listed in Tables 3 and 4); *lane 4*, normal lymph node; *lane 5*, CEM; *lane 6*, MT-2; *lane 7*, MOLT-4F. The *arrow* indicates 1.3 kb. Intactness of total RNA was confirmed by agarose gel electrophoresis following denaturation with glyoxal

Table 5. Relationship between expression and rearrangement of T-cell receptor β gene in TCRβ−CD3+ T-cell lymphomas

Case no.	Surface markers			Expression of TCRβ mRNA	Rearrangement of TCRβ gene
	TCRβ	CD3	CD25		
13	−	+++	++	−	R[a]
14	−	++	+++	−	R
15	−	+++	+++	Not tested	R

[a] R, rearrangements

tumour of the lymphocyte predominance type, while the expression was negative for all other types.

Northern blot analysis was performed on polyadenylated RNA to analyse the transcription of the TCRβ gene of βF1 negative T-cell lymphomas in which TCRβ gene rearrangement was observed. Full length TCRβ transcripts of 1.3 kb were demonstrated in T-cell leukaemic cell lines such as MOLT-4F, MT-2 and CEM, normal lymph node and Hodgkin's disease. However, no transcripts of TCRβ were observed in 2 βF1 negative T-cell lymphomas in which there was TCRβ gene rearrangement (Fig. 6, Table 5).

Discussion

The immunoreactivity of anti-TCRβ chain mAb, βF1, in T-cell lymphomas was investigated and 3 cases of βF1−CD3+ T-cell lymphomas were observed. βF1−CD3+ T-cell lymphomas have been reported previously but without any indication as to the molecular basis for aberrant expression of TCRβ in surgical material from βF1−CD3+ T-cell lesions (Chan et al. 1988; Ng et al. 1988). The expression of TCR in T-cells requires a complete functional rearrangement to encode for mRNA and co-expression of CD3 molecules in order to make functional units in T-cells (Minden and Mak 1988). TCRβ gene rearrangement in the 3 cases in the present study was not accompanied by TCRβ gene transcripts in 2 cases. The lack of transcripts in βF1−CD3+ T-cell lymphomas may thus be considered responsible for the non-expression of TCRβ protein. The non-transcription of the TCRβ gene in T-cell malignancies can be explained on the basis of structural abnormalities of genes or non-productive rearrangement, possibly associated with neoplastic transformation or alteration (Duby and Seidman 1986; Su et al. 1988). TCRβ transcripts and protein were shown to be induced by phorbol ester in βF1−CD3− post-thymic T-cell lymphoma cell lines which rearranged the TCRβ gene locus. Thus, the functional rearrangement of TCR genes in those T-cell lines may occur (Su et al. 1988). Although it could not be determined whether TCRβ gene rearrangements in βF1−CD3+ T-cell lymphomas were non-productive or functional in the present study, the loss of one or more T-cell antigens in peripheral T-cell lymphomas has been observed in lymphomas of high-grade malignancy (Stein et al. 1984; Hollema and Poppema 1989) and reported to be accompanied by the expression of activation antigens such as IL-2R or HLA-DR (Winberg et al. 1985; Borowitz et al. 1986; Mastuoka et al. 1986). In the present study, 3 βF1−CD3+ T-cell lymphomas including 1 ATL were high-grade and expressed IL-2R to a high degree. The absence of the T-cell antigen and expression of activation antigens are considered to be related to tumour progression or activation processes of T-cells.

The expression of TCRβ and TCRδ in lymphoid tissues and malignant lymphomas was examined immunohistochemically and TCRβ expression was noted in most T-cells of the thymus-dependent areas in normal lymphoid tissues such as thymus, lymph nodes and ton-

sil, and most peripheral T-cell lymphomas. In contrast to TCRβ, TCRδ was expressed in a small sub-population of normal T-cells in lymphoid tissues and did not react with malignant lymphoma cells. With regard to other surface markers, TCR and CD3 were co-expressed in the lymphoid tissues and most peripheral T-cell lymphomas including 13 CD3+4+8− cases and 2 CD3+4−8+ cases, although TCR heterodimers were associated with CD3 proteins in normal T-lymphocytes. Essentially the same findings have been reported for the expression of TCR and CD3 (Chan et al. 1988; Ng et al. 1988). As for the expression for TCRβ and CD3 in post-thymic T-cell malignancies, four different patterns, i.e., βF1+CD3+, βF1−CD3+, βF1+CD3− and βF1−CD3−, were observed (Ng et al. 1988). The major immunophenotype of peripheral T-cell lymphomas was βF1+CD3+, which is considered to represent classical T-cell immunophenotype. Although βF1+CD3− was shown to be an aberrant T-cell immunophenotype, 1 Ki-1 lymphoma was classified as this phenotype. The immunophenotype of 3 T-cell lymphomas was βF1−CD3+. Although Ng et al. (1988) considered that it was possible that CD3+TCRβ− T-cell tumours represent the malignant counterparts of normal T-cells expressing TCR$\gamma\delta$, the present 3 cases of TCRβ−CD3+ T-cell lymphomas (cases 13–15) were negative for TCRδ. The immunophenotype of 1 TCRβ-TCRδ-lymphoblastic lymphoma was CD1−CD3−CD4−CD7+CD8− and thus this phenotype may represent the malignant counterpart of T-cell precursors not expressing CD3 and TCR molecules (Haynes et al. 1988).

The expression of TCR protein was examined in Hodgkin's disease. Hodgkin's and RS cells were negative for βF1 and TCRδ1, although the expression of T-cell antigens such as T11, Leu3 and Leu4 in RS cells has been reported (Kadin et al. 1988). Southern blot analysis of 2 Hodgkin's disease cases showed diminished intensity of the 11-kb band of EcoRI digestion using TCRβ probe, possibly indicating a polyclonal T-cell population (Roth et al. 1988). A single band of 1.3 kb was found in one Hodgkin's disease case by Northern blot analysis and considered to have derived from non-neoplastic polyclonal T-cells but not RS cells. The cellular origin of RS cells could not be determined in the present study.

TCR and Ig gene rearrangements have been found to function as markers of clonality and the cell lineage of lymphoproliferative disorders (Minden and Mak 1986; Feller et al. 1988). However, they do not indicate cell lineage in all cases, since both rearrangements may cross lineage lines and neither are they restricted to B- or T-cells (Cossman et al. 1988). The analytical results of these rearrangements have been found applicable to the monitoring of therapy in lymphomas and to facilitate the early detection of lymphoma recurrence (Minden and Mak 1986). All T-cell lymphomas examined in the present study were shown to exhibit rearranged TCRβ gene but no rearrangement of the Ig J_H gene, thus confirming the clonality of these neoplasms. The clonality for T-cells could not be determined by phenotypic markers and consequently, to facilitate the diagnosis of lymphoproliferative disorders, the examination of gene rearrangement and immunophenotyping should be conducted together.

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