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ORIGINAL ARTICLE

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Restricted Vβ gene usage of tumour-infiltrating T lymphocytes in primary gastric malignant B-cell lymphoma

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Abstract Ten cases of primary gastric malignant lymphoma (PGL) were investigated by immunohistochemical and molecular genetic analysis. These cases were diagnosed histopathologically as follicular small cleaved cell type (1 case), diffuse small cleaved cell type (3 cases) and diffuse large cell type (6 cases) based on the WF (Working Formulation) classification. Seven cases classified as small cleaved or diffuse large cell type belong to low (4 cases) or high (3 cases) grade MALT lymphoma according to Isaacson's classification. All PGL belonged to B lineage cells according to immunohistochemical study and immunoglobulin rearrangements. Rearrangements of TCR β chain genes were observed in four of the ten cases. The possibility that the TCR β rearrangements were caused by tumour-infiltrating T-cells (TILs) was supported by the following observations: the tumours did not show T- and B-cell biphenotype, TCR β exhibited functional VDJ rearrangement and Vβ usage pattern was not a neoplastic type. Analysis of the repertoire of the TCR β chain in TILs revealed a common usage of Vβ2 in the above four cases, and furthermore, predominant usage of a particular β chain composed of Vβ2-Dβ2.1-Jβ2.3 was observed in one of the four cases. These results indicate that the TILs of PGL have a restricted TCR repertoire.

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Introduction

Analysis of the lineage and differentiation stage of neoplastic cells is crucial for the diagnosis of malignant lymphomas. Rearrangement analysis of immunoglobulin (Ig) and T cell receptor (TCR) genes has also emerged as a useful diagnostic method for this purpose [9, 13, 21]. In general, T and B cells exhibit rearrangement of TCR and Ig, respectively. However, it has been shown in a minority of cases that both TCR and Ig gene rearrangements occur in the same tissues or cell populations [15]. In most of these cases, the tumours belong to immature B or T cells [7, 16, 34], and the rearrangements are often incomplete or not transcribed [10, 12].

The genomic organization of TCR β genes is composed of at least 57 variable, 2 diverse, 13 joining and 2 constant regions. The TCR Vβ genes have been divided into 24 families on the basis of sequence homology [32]. Whereas the repertoire of TCR β is heterogeneous in normal peripheral T cells, a limited repertoire has been reported in some cases of T cell responses to particular antigens as well as in tumour-infiltrating lymphocytes (TILs). When the TCR usage of reactive T cells is limited, it is conceivable that the immune response is modulated by the inactivation of these T cells.

In the course of genotypic analysis of primary gastric lymphomas (PGL), we encountered four cases that exhibited rearrangements of both TCR and Ig. Phenotype and genotype analysis indicated that the TCR rearrangement represented an oligoclonal expansion of reactive T cells infiltrating the lymphoma. These TILs showed restricted usage of Vβ genes and, in one case, a particular junctional sequence. These results are discussed in relation to recent reports that TILs in some tumours have restricted usage of V genes.

Materials and methods

Specimens were obtained from patients who were clinically diagnosed as having PGL and underwent resection. A portion of the specimen was snap-frozen in liquid nitrogen for immunohistochemical and genotypic analysis.

Routinely processed formalin-fixed, paraffin-embedded samples were sectioned at 3 μm and stained with haematoxylin-eosin. Classification of non-Hodgkin's lymphomas was based on the Working Formulation (The non-Hodgkin's lymphoma pathological classification project [39]) and Isaacson's classification [19].

The immunological phenotype of each PGL was determined by immunohistochemical methods using the standard avidin-biotin complex technique [17], and the results were explained in conjunction with those from the histopathological studies. The antibodies used are shown in Table 1.

Lymphomas were classified as B-cell lineage when neoplastic cells were stained with the following B-cell specific antibodies: CD19, CD20, CD21 or CDw76; they were thus not classified when stained with these anti-T cell antibodies: CD45RO, CD43. CD3, CD4, CD5, CD8 or βF1. On the contrary, they were identified as T-cell lineage when neoplastic cells showed positive staining with T-cell markers but were negative with B-cell markers.

Table 1 Monoclonal antibodies used in this study

Antibody	Antigen	Source
Pan-B-cell		
B1	CD20	Coulter Immunology
B2	CD21	Coulter Immunology
B4	CD19	Coulter Immunology
LN-1	CDW75	Nichirei
L26	CD20	Dakopatts
Pan-T-cell		
Leu1	CD5	Becton Dickinson
Leu2a	CD8	Becton Dickinson
Leu3a	CD4	Becton Dickinson
Leu4	CD3	Becton Dickinson
UCHL-1	CD45RO	Dakopatts
MT-1	CD43	Bio-Science
βF1	TCRβ chain	T cell Science

Double staining with CD45RO and CD20 antibodies was performed in some cases in order to determine whether there was simultaneous expression of T and B markers on neoplastic lymphocytes.

For gene rearrangement analysis DNA and RNA were isolated by the method of Maniatis et al. [22]. Tissues were homogenized mechanically and centrifuged at 35000 rpm for 18 h at 20° C by the guanidinium/caesium chloride method. Isolated genomic DNA was incubated for 2 h at 50° C with 200 μg/ml proteinase K, and 0.5% sodium dodecyl sulphate, extracted by phenol/chloroform, and subsequently dialysed.

For Southern blot analysis, 10 μg of DNA was digested with restriction enzyme *Bam*HI, *Eco*RI or *Hind*III, electrophoresed on 0.8% agarose gel, and transferred to nylon membranes (Hybond N., Amersham Japan, Tokyo). The blots were hybridized with ^{32}P-dCTP-labelled immunoglobulin (J_H, C_K) and TCR (Cβ) cDNA probes for at least 16 h under high-stringency conditions at 65° C, washed with a final stringency of 0.2× sodium salt citrate (SCC) (1×SSC is 0.15 M-sodium chloride, 0.015 M-sodium citrate) at 65° C, and subjected to autoradiography.

A first-strand cDNA was synthesized from 5 μg of total RNA in a 20 μl reaction mixture containing oligo(dT) primer and AMV reverse transcriptase (RT) according to the manufacturer's protocol (Amersham). A 1 μl sample of first-strand cDNA (corresponding to 250 ng of total RNA) was subjected to PCR amplification with 2.5 units of *Taq* polymerase (Perkin-Elmer) in 100 μl of standard buffer with 20 different Vβ-specific primers and a Cβ primer at a final concentration of 0.25 μM in each reaction to detect each Vβ gene (Table 2). These primers were previously used for normal peripheral T cells as well as autoimmune patients. Although the Cβ primer was different from that used previously (Sumida et al. [35]), the results obtained by preliminary experiments showed that all the primers could be used for amplification with similar efficiency. The denaturing step was carried out for 90 s at 94° C, the annealing step for 90 s at 60° C, and the extention step for 60 s at 72° C for 30 cycles by using a Thermal Sequencer (Iwaki Glass, Japan).

One twenty-fifth of the PCR products was electrophoresed on 2.0% agarose gel, stained with ethidium bromide, and then hybridized with a Cβ probe for Southern blot analysis. Amplified DNA bands (360–390 bp) bearing VDJC regions of β chain gene were detected.

For DNA sequencing, PCR fragments were digested with *Eco*RI and the fragments were subcloned into the *Eco*RI site of M13mp19. The sequencing was performed by the dideoxynucleotide chain termination method (Sanger et al. [33]).

Table 2 Oligonucleotide primers used for PCR. The size of amplified products (Vβ bands) with 5′ Vβ and 3′ Cβ primers ranged from about 360 to 390 bp

Primer	5′→3′ Sequence										
Vβ1	TCT	AGA	ATT	CCA	AAA	GGA	AAC	ATT	CTT	GAA	C
Vβ2	TCT	AGA	ATT	CCC	ACA	TAC	GAG	CAA	GGC	GTC	G
Vβ3	TCT	AGA	ATT	CGA	AAA	AGG	AGA	TAT	TCC	TGA	G
Vβ4	TCT	AGA	ATT	CCA	TAT	GAG	AGT	GGA	TTT	GTC	A
Vβ5	TCT	AGA	ATT	CAA	AGG	AAA	CTT	CCC	TGG	TCG	A
Vβ6	TCT	AGA	ATT	CAG	ATG	ACT	CAG	GGC	TGC	CCA	A
Vβ7	TCT	AGA	ATT	CAG	TGT	GCC	AAG	TCG	CTT	CTC	A
Vβ8	TCT	AGA	ATT	CAT	AGA	TGA	TTC	AGG	GAT	GCC	C
Vβ9	TCT	AGA	ATT	CTG	AAA	CAG	TTC	CAA	ATC	GCT	T
Vβ10	TCT	AGA	ATT	CAA	AGC	AGA	AAT	AAT	CAA	TGA	G
Vβ11	TCT	AGA	ATT	CAA	GGG	AGA	TCT	TTC	CTC	TGA	G
Vβ12	TCT	AGA	ATT	CAA	AGG	AGA	AGT	CTC	AGA	TGG	C
Vβ13	TCT	AGA	ATT	CAT	GGC	TAC	AAT	GTC	TCC	AGA	T
Vβ14	TCT	AGA	ATT	CAA	GGG	AGA	TGT	TCC	TGA	AGG	A
Vβ15	TCT	AGA	ATT	CAA	AGG	AGA	GAT	CTC	TGA	TGG	A
Vβ16	TCT	AGA	ATT	CCT	TTA	TCG	ACG	TGT	TAT	GGG	A
Vβ17	TCT	AGA	ATT	CGG	AGA	TAT	AGC	TGA	AGG	GTA	C
Vβ18	TCT	AGA	ATT	CGG	AAT	GCC	AAA	GGA	ACG	ATT	T
Vβ19	TCT	AGA	ATT	CGA	GAT	GCA	CAA	GAA	GCG	ATT	C
Vβ20	TCT	AGA	ATT	CCT	GCA	GGC	AGG	GGC	CTC	CAG	C
Cβ	TCT	AGA	ATT	CTC	TGT	GCT	GAC	CCC	ACT	GTG	C

Results

The histopathological features of the biopsy samples used in this study are shown in Table 3. All of ten cases were classified as B-cell lymphoma on the basis of the expression of at least two of the four B-cell markers tested – CD19, CD20, CD21 and CDw75 antigens. Two-colour immunostaining with CD20 and CD45RO antibodies showed that tumour cells did not express the T-cell phenotype in the four cases tested (Fig. 1). In cases 4, 7 and 9, large numbers of small T lymphocytes were found to infiltrate the tumour (Table 4).

Southern blot analysis with a J_H probe showed one (four cases) or two (four cases) rearranged bands in addition to the germ-line one (Fig. 2, Table 3). In case 7, three and two rearranged bands were detected by J_H and C κ probes, respectively, suggesting that DNA was derived from at least two independent clones. In another case (case 4), only κ rearrangement was observed together with the germ-line configuration of the IgH gene.

Table 3 Non-Hodgkin's gastric lymphomas cases studied (*F*, female; *M*, male; *B*, B cell lymphoma; *T*, T cell lymphoma; *R*, rearranged; *G*, germ line)

Cases	Age/Sex	Histology (Working Formulation, 1982) (Isaacson, 1992)	Phenotype	Genotypic analysis				
				J_H (*Eco*RI)	C_κ (*Bam*HI)	TCRβ (*Hin*dIII,	*Eco*RI	*Bam*HI)
1	61/F	Diffuse large[c]	B	R	R	R	R	R
2	74/M	Follicular small cleaved Low-grade MALT	B	2R	G	G	G	G
3	72/F	Diffuse large High-grade MALT	B	R	R	G	G	G
4	50/M	Diffuse small cleaved Low-grade MALT	B	G	R	3R	R	R
5[a]	59/F	Diffuse unclassified[b]	T	G	G	R	R	R
6	44/F	Diffuse large[c]	B	2R	G	G	G	G
7	?/F	Diffuse large High-grade MALT	B	3R	2R	R	R	R
8	61/M	Diffuse small cleaved Low-grade MALT	B	R	R	G	G	G
9	44/M	Diffuse large High-grade MALT	B	2R	R	R	R	R
10	?/F	Diffuse large[c]	B	2R	R	G	G	G
11	45/M	Diffuse small cleaved Low-grade MALT	B	R	G	G	G	G

[a] Case 5 is T-cell lymphoma of the duodenum used as control

[b] Diffuse lymphoma of medium-sized cells with occasional convoluted nuclei, which was difficult to classify according to Working Formulation

[c] Diffuse large lymphoma without evidences of MALT lymphoma

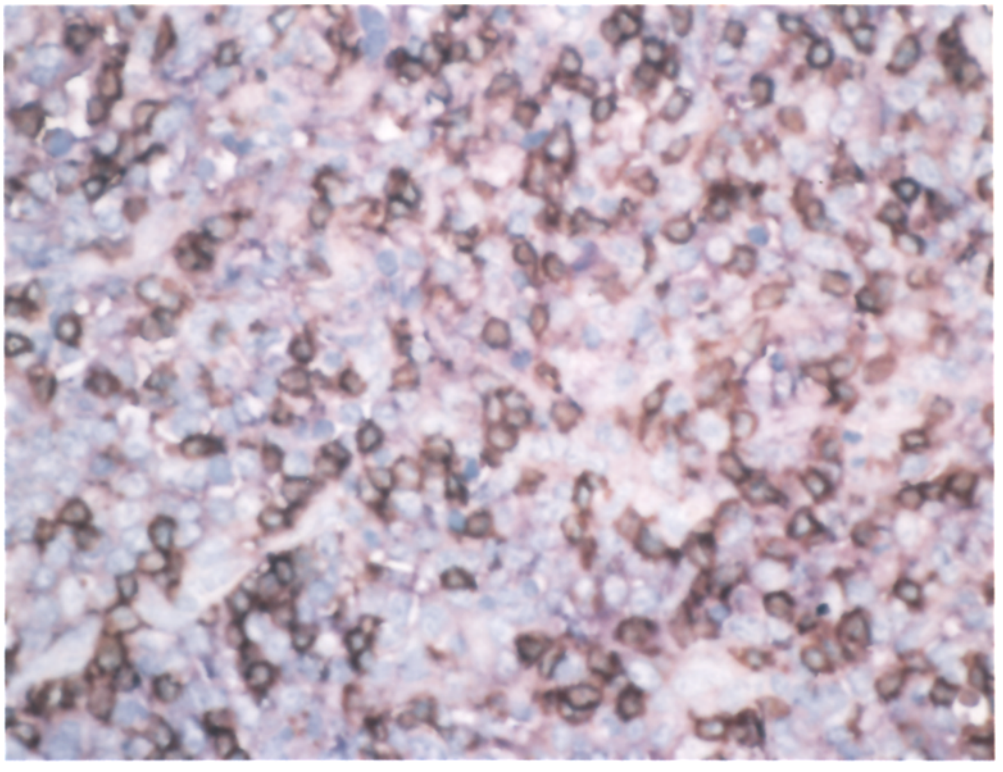

Fig. 1 Section of a primary gastric malignant lymphoma (PGL) (case 9) doubly stained for CD45RO(UCHL-1) and CD20(L26). T- and B-cells were stained brown with CD45RO and violet with CD20, respectively. Tumour cells were large with large nuclei and distinct nucleoli and stained violet with CD20, while smaller infiltrating T cells were stained brown. No tumour cells showed both colours (black). 575

Table 4 Immunophentoypic characteristics of gastric lymphoma cells ( ), indicates reactions of infiltrating cells; *ND*, not done)

mAb	Case:	1.	2.	3.	4.		6.	7.	8.	9.	10.	11.
	P.D.	AS.61F DL	SY.74M FSC	NT.72F DL	HY.50M DSC		ST.44F DL	OT...F DL	KS.61M DSC	KM.44M DL	IY...F DL	IN.45M DSC
					1	2						
Paraffin section												
CD20 (L26)		++	++	++	(+)	++	++	++	++	++	++	++
CDW75 (LN-1)		++	+	++	(+)	++	ND	++	++	+	+	+
CD45RO (UCHL-1)		(+)	(+)	(+)	(++)	(+)	(+)	(+)	(+)	(++)	(+)	(+)
CD43 (MT1)		(+)	(−)	(+)	(++)	(+)	(+)	(+)	(+)	(++)	(+)	(+)
Frozen section												
CD20 (Leu16)		++	ND	−	(+)	++	ND	++	++	++	++	++
CD21 (B2)		+	ND	+	(+)	+	ND	ND	ND	ND	+	+
CD19 (B4)		++	ND	−	(+)	++	ND	++	++	++	++	++
CD5 (Leu1)		(+)	ND	(+)	(++)	(+)	ND	(++)	(+)	(+)	ND	ND
CD8 (Leu2a)		(+)	ND	(+)	(+)	(+)	ND	(+)	(+)	(++)	(+)	(+)
CD4 (Leu3a)		(+)	ND	(+)	(+)	(+)	ND	(+)	(+)	(+)	(+)	(+)
CD3 (Leu4)		(+)	ND	(+)	(++)	(+)	ND	(++)	(+)	(++)	(+)	(+)
TCRβ (βF1)		(+)	ND	(+)	(++)	(+)	ND	(++)	(+)	(++)	(+)	(+)

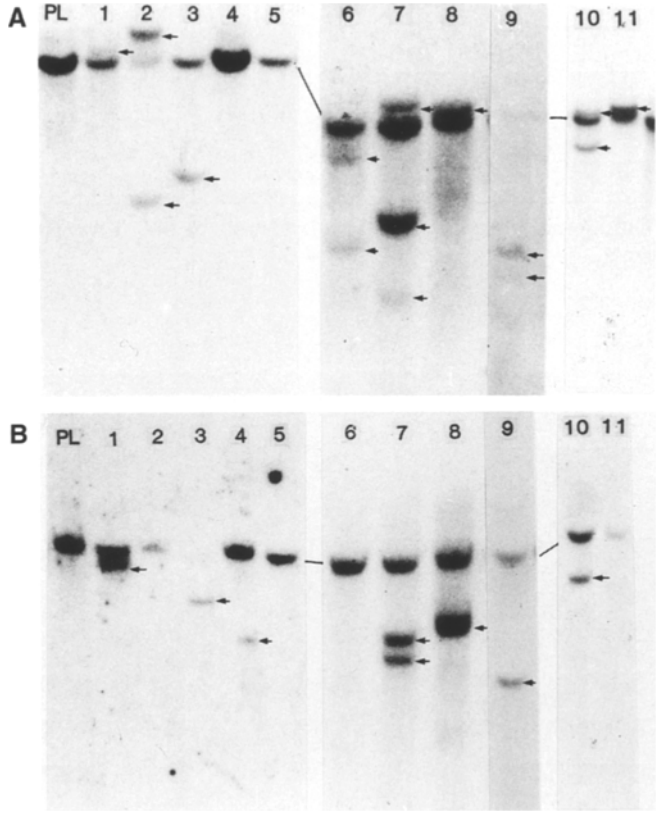

Fig. 2 Southern blot analysis of rearrangement of the Ig genes in PGL. Rearrangement bands are indicated by *arrows* (*PL*, germ-line control). The number above each of the other lanes corresponds to the case number in Table 3. (**A**) DNA rearrangement of the IgH chain gene using the J_H probe after *Eco*RI digestion. Lane 7, case 7 (Table 3) shows three rearranged fragments that correspond to at least two separate clones. Lane 4, case 4 (Table 3) shows the germ-line configuration without rearrangement bands. Other cases show the rearrangement of one or two alleles. (**B**) DNA rearrangement of the Ig_K chain gene using the C_K probe after *Bam*HI digestion. Lanes 1, 3, 4, 8, 9 and 10 show one rearranged fragment. Lane 7 shows two rearranged bands

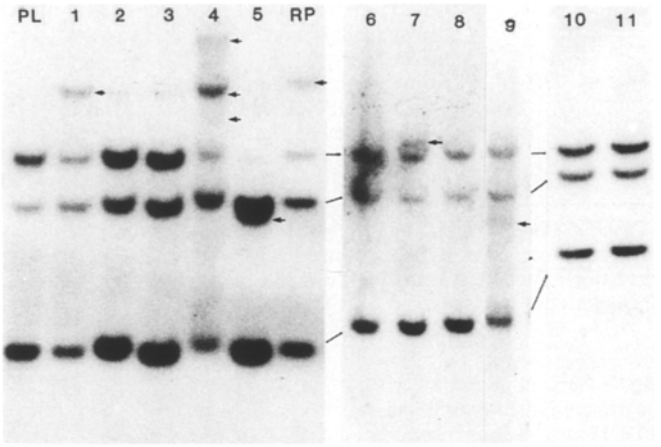

Fig. 3 Southern blot analysis of rearrangement of TCR β chain gene using the Cβ probe after *Hind*III digestion. Rearrangement bands are indicated by *arrows*. (PL, germ-line control) RP, T-cell line served as positive control. The number above each lane corresponds to the case number in Table 3. Lanes 1, 7 and 9 show one rearranged band. Lane 4 shows three rearranged bands. The intensity of these rearrangement bands is lower than that of lane 5, a T-cell lymphoma of the duodenum (used as control)

As for the TCR genes, four cases displayed rearrangements of both TCR β and Ig genes(Fig. 3, Table 3). Since these rearrangement bands were demonstrated by all three restriction enzymes, incomplete digestion was ruled out. In three of them, many T lymphocytes infiltrated the tumour tissue. In case 9, most of the infiltrating T-cells were $CD8^+$ phenotype (Table 4).

Based on the assumption that the observed rearrangements of the TCR β genes in these four cases were derived from TIL, the TCR β repertoire was examined by RT-PCR. mRNA was isolated from tumour tissues, and reverse-transcribed. Single strand cDNA was amplified by PCR using a combination of one of the primers spe-

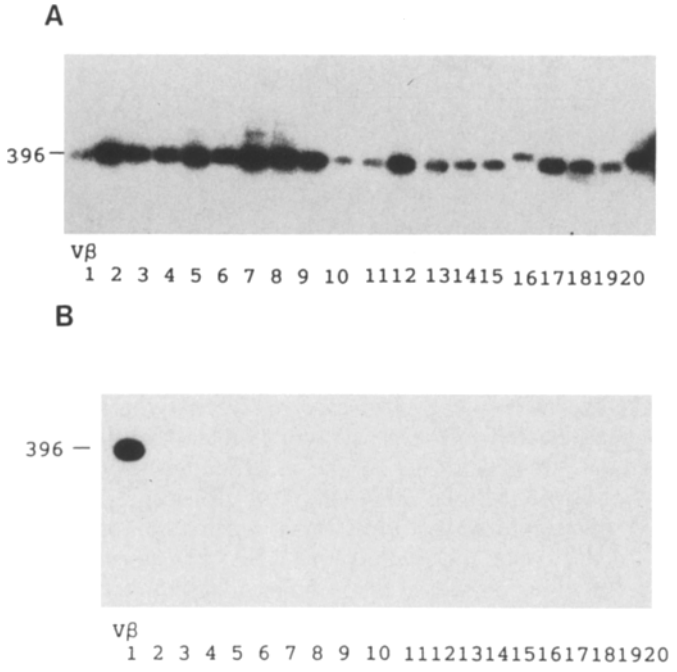

Fig. 4 Southern blot analysis of PCR-amplified products encoding TCR Vβ on a normal PBLs and a primary duodenal T-cell lymphoma (control). cDNA was synthesized with total RNA (10 μg) and was amplified for 30 cycles with 20 different Vβ primers (Table 2). The amplified products were electrophoresed on 0.2% agarose gel and hybridized for Southern blot analysis with the ^{32}P-labelled Cβ probe. (**A**) Detection of PCR-amplified products from PBLs of a healthy individual. All Vβ-containing TCR β chains could be detected. (**B**) In a case of primary duodenal T-cell lymphoma, the Vβ1 amplified product shows a single intense band that corresponds to the tumor clone.

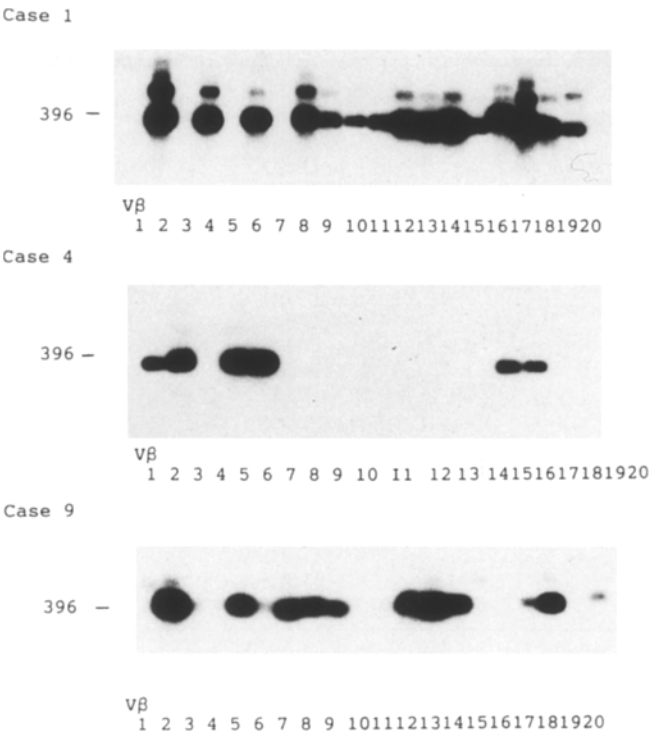

Fig. 5 Representative PCR results with the Vβ primers on PGL. Southern blot analysis of PCR-amplified products is shown for cases 1, 4 and 9. All cases show several dominant bands. Vβ2 is expressed in common in each case. Vβ2, 4 and 5 are predominantly expressed in case 4. Vβ2 has the highest expression in cases 1 and 9

cific for 20 different human TCR Vβ families and a common Cβ primer. It was shown that all TCR Vβ were detected in peripheral T cells by using the same primers (Fig. 4A). In addition, when a duodenal T-cell lymphoma was used as a control of monoclonal T-cells, a single major Vβ1 band was observed (Fig. 4B). In contrast, the Vβs used in these four B-PGL cases were multiple, with hybridized bands of various intensities. Unlike the duodenal T-cell lymphoma, no single dominant Vβ band was observed.

Representative patterns of Vβ usage are shown for cases of 1, 4 and 9 in Fig. 5, with restricted usage being especially evident in case 4. In this case, three rearranged bands of TCRβ gene were observed by Southern blot analysis (Fig. 3) and three Vβs, Vβ2, 4 and 5 were detected by PCR (Fig. 5). Vβ2 was expressed in all four cases (Table 5). Sequencing of the Vβ2 products in these cases showed the dominant expression of a particular rearrangement in cases 1 and 9 (Table 6). Analysis of 12 clones revealed that the Vβ2 repertoire in case 1 contained 6 distinct rearrangements, and one particular rearrangement (Vβ2 Dβ Jβ2.7) was observed in 6 clones. In case 9, the Vβ2 Dβ2.1 Jβ2.3 rearrangement was found in 6 of 9 clones. The N region sequence of the predominantly used β chain was exactly the same among the clones. On the other hand, Vβ2 was not rearranged to any unique Dβ and Jβ in cases 4 and 7 from the sequence analysis of 5 clones each.

Table 5 Usage of TCR Vβ gene in primary gastric lymphoma (More intense expressions by hybridized Vβ genes in each case were indicated by bold face)

Case No.	Vβ families											
1		**Vβ2**	Vβ4	Vβ15		Vβ6 Vβ16	Vβ8 Vβ17	Vβ9 Vβ18	Vβ10 Vβ19	Vβ11	Vβ12	Vβ13	**Vβ14**
4	Vβ1	**Vβ2**	**Vβ4**	**Vβ5** Vβ15									Vβ14
7		**Vβ2**		Vβ15	Vβ5	Vβ7	Vβ8	Vβ9 Vβ18				Vβ13	
9		**Vβ2**			Vβ5	Vβ7	Vβ8	Vβ9 Vβ18			Vβ12	**Vβ13**	Vβ14

Table 6 TCR sequences of Vβ2 PCR products (The shading indicates dominance of a particular rearrangement)

Case No.			ratio
1	Vβ2	D-Jβ1.1	1/12
		D-Jβ1.2	1/12
		D-Jβ2.1	1/12
		D-Jβ2.2	1/12
		D-Jβ2.3	2/12
		D-Jβ2.7	**6/12**
4		D-Jβ1.1	1/5
		D-Jβ1.5	1/5
		D-Jβ2.1	1/5
		D-Jβ2.3	1/5
		D-Jβ2.7	1/5
7		D-Jβ1.2	1/6
		D-Jβ2.1	1/6
		D-Jβ2.4	1/6
		D-Jβ2.5	1/6
		D-Jβ2.7	2/6
9		D-Jβ1.1	2/9
		D-Jβ1.2	1/9
		[a] **D-Jβ2.3**	**6/9**

[a] D is Dβ2.1

Discussion

Analysis of the rearrangement of Ig and TCR genes is a useful procedure for studying the clonality and lineage of malignant cells in lymphoproliferative disorders. However, the results must be interpreted with great care. For example, the abortive D-J rearrangement of IgH and TCRβ genes can be found in malignant cells belonging to B-, T- or myeloid cells [5, 24, 37]. Furthermore, some B-cell lymphomas have been shown to exhibit rearrangements of TCRγ and/or TCRδ chain genes [14, 40]. Tumours thought to be of "dual" genotype and containing rearrangements of both Ig and TCR genes appear to be more frequent in B-cell than T-cell neoplasms [30].

In the present study, we found rearrangement of both Ig and TCRβ genes in four of ten B-PGLs. This frequency is much higher than that previously reported for B-cell lymphomas [2, 7, 10]. These four cases were considered histologically to be diffuse malignant lymphoma of B-cell lineage, since all tumour cells expressed at least two B antigens and showed rearrangement of IgH and/or Ig-κ These findings also suggest that the tumour clones were derived from mature B lymphocytes. The rearranged TCRβ genes observed in these cases were functional, as shown by the presence of 1.3 kb RNA transcripts (data not shown) and subsequently by sequencing of the TCRβ transcripts. Further, the complete TCRβ gene rearrangement has not been reported in mature B-cell neoplasms.

The failure of double immunostaining in the present study showed that the tumours were not biphenotypic in these four cases. Furthermore, three of the four contained large numbers of T lymphocytes admixed with neoplastic B-cells. From these results, we assumed that the rearrangements of TCRβ gene may reflect the presence of TILs rather than tumour cells. The fact that the intensity of the rearranged TCRβ gene bands was always weaker than that of the J_H bands by Southern blot analysis suggested that the rearrangement of TCRβ gene was not derived from the malignant B-cell clone but rather reflected the minor population of reactive T lymphocytes. Some of the previous reports describing "dual" genotypic lymphomas may have actually studied the infiltrating T cells instead of tumour cells as in the present study.

Our study also demonstrated that analysis of the Vβ repertoire is able to distinguish whether the genotype analysed represents normal or neoplastic T lymphocytes. As shown in the control case of T-cell lymphoma, Vβ gene detected in the T-cell lymphoma is restricted to a single Vβ gene, whereas the reactive T-cell population showed oligoclonal dominancy for Vβ usage. Bahler et al. [4] suggested that in most biopsy material, malignant T-cells would contribute more RNA than normal T-cell populations present in the samples, and that therefore the clonality has to be determined by Southern blotting. The TCR β chains detected in our four cases were not a single β chain but contained several Vβ usages in each case. We used 20 different Vβ primers for PCR amplification and these were shown to amplify all Vβ-containing TCR β chains from peripheral T cells. The cDNAs of normal PBLs encoding 20 different Vβ genes were quantitated by individual standardization curves that represented the relationship between amount of cDNA used for PCR, and the radioactivity of the PCR product was plotted in a log-log graph against the amount of cDNA(RNA) encoding each individual Vβ gene. A linear relation was obtained, as in previous report [35]. The angles of inclination were similar with all probes. Consequently, the minimum sensitivity of the detection of our PCR was about $3–10^2$ (RNA)pg (data not shown). In addition, when three lymph nodes were used as reactive lymphoid tissues, various Vβ genes were amplified other than Vβ2 [3]. Considering the fact that only one Vβ was amplified from T-cell lymphoma (control case) with these primers, the restricted repertoire is not apparently the result of experimental limitations. Therefore, it is likely that the amplified β chains in each of the four cases represent those in the TIL populations. It is noteworthy that three rearranged bands of TCRβ were observed in case 4 by Southern blot analysis, and three Vβ usages (Vβ2, Vβ4, Vβ5) were detected by RT-PCR. This readily leads to the assumption that the three amplified Vβ genes correspond to the three rearranged bands by Southern blot analysis. In fact, the Vβ2 probe revealed hybridization with one of these rearranged bands (data not shown).

Increasing numbers of studies have reported on restricted TCR β usage in various diseases, such as experimental allergic encephalomyelitis [1, 42], allograft rejection [6, 23], multiple sclerosis [29], Grave's disease [11, 38], sarcoidosis [26], primary biliary cirrhosis [25], Crohn's disease [31] and choriomeningitis [41]. It is also well known that TCR V gene usage is restricted in human T cells stimulated with superantigen [8, 20] as well

as in TIL of melanoma and glial tumours [27, 28]. Our findings expand these observations to include human PGLs.

Our results also indicate that TILs in some cases of PGL of the stomach possess specific rearrangements containing Vβ2 Dβ and Jβ. In case 9, in which a large number of $CD8^+$ lymphocytes infiltrated the tumour, Vβ2 predominantly rearranged to Dβ2.1 and Jβ2.3. Such restricted junctional diversity was also suggested by the fact that the sequence of the N region was identical. It has been suggested that VDJ junctional regions are important in the recognition of antigenic peptides in general as well as in TIL. Suzuki [36] et al. reported that T-cells from enlarged lymph nodes of Friend virus-induced mice erythroleukaemia dominantly expressed homogeneous TCRβ chain gene with a particular junctional region. Our observations regarding the TCR Vβ2 gene were consistent with these reports and might suggest the existence of specific antigen(s). Recently, Hussell [18] et al. reported that low grade MALT gastric lymphoma might be related to *Helicobacter pylori* infection. In PGL, *H. pylori* may also act as a specific antigen to stimulate subsets of T-cell with restricted Vβ usage.

In the present study, we showed the restricted usage of TCRβ chain gene in infiltrating T-cells in primary gastric B cell lymphoma for the first time. Whether there exists a specific antigen corresponding to the restricted T-cell receptor remains to be determined.

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