Jpn. J. Cancer Res. **92**, 1041–1047, October 2001

DNA Sequence of Immunoglobulin Heavy Chain Variable Region Gene in Thyroid Lymphoma

Hideaki Miwa,[1] Tetsuya Takakuwa,[1] Shin-ichi Nakatsuka,[1] Yasuhiko Tomita,[1] Fumio Matsuzuka[2] and Katsuyuki Aozasa[1, 3]

[1]*Department of Pathology, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871 and* [2]*Department of Surgery, Kuma Hospital, Kobe 650-0011*

Patho-epidemiological studies have shown that thyroid lymphoma (TL) develops in thyroid affected by chronic lymphocytic thyroiditis (CLTH). CLTH is categorized as an organ-specific autoimmune disease, in which activated B-lymphocytes secrete a number of autoantibodies. Because antigenic stimulation might be involved in the pathogenesis of TL, the variable region in heavy chain (V_H) genes was characterized in 13 cases with TL and 3 with CLTH. Clonal rearrangement of the V_H gene was found in 11 cases of TL, and cloning study with sequencing of complimentarity determining region (CDR) 3 revealed the presence of a major clone in 4. Three of the 4 cases used V_H3 gene, with the homologous germline gene of *V3-30* in two cases and *VH26* in one case. A biased usage of V_H3 and V_H4 genes with the homologous germline gene of *VH26* in V_H3 gene was reported previously in cases with CLTH. A high level of somatic mutation (1–21%, average 12%) with non-random distribution of replacement and silent mutations was accumulated in all cases. The frequency of the occurrence of minor clones ranged from 29–44% per case, indicating the presence of on-going mutation. DNA sequencing of immunoglobulin V_H gene suggests that TL develops among activated lymphoid cells in CLTH at the germinal center stage under antigen selection.

Key words: Thyroid lymphoma — DNA sequence — *Ig* V_H gene — Hashimoto's disease

Thyroid lymphoma (TL) is B-cell lymphoma,[1)] and a minor constituent of non-Hodgkin's lymphoma, accounting for 2.5% of all cases of extranodal lymphomas in the series of Freeman and associates from North America[2)] and 2.2% in our series from Japan.[3)] TL had attracted the attention of investigators because of its putative origin from active lymphoid cells in autoimmune thyroiditis, i.e., Hashimoto's thyroiditis or chronic lymphocytic thyroiditis (CLTH).[4)] Lymphoid cell infiltration forming lymphoid follicles with hyperplastic germinal centers is a consistent finding in CLTH and this is frequently observed even in TL. Follow-up studies from Sweden[5)] and Japan[6)] confirmed an important role of CLTH in the development of TL: the frequency of TL is 67–80 times higher in patients with CLTH than in age- and sex-matched normal individuals. CLTH is categorized as an organ-specific autoimmune disease, in which activated B-lymphocytes from thyroid tissue of patients secrete a number of autoantibodies directed against thyroid antigens, such as thyroglobulin, thyroid-stimulating hormone (TSH) receptor, and iodine transporter.[7)] Thus, antigen-selected proliferation of B-cells might take place in the lesional thyroid tissues of TL.

The diversity of lymphocytes depends on about 200 genes, which are called variable-region genes. The variable region of heavy chain consists of appoximately 100 *V* genes, 30 *D* genes, and 6 *J* genes, but only one of each kind is used to assemble the *V-D-J* coding sequence. In the course of antigen-specific immune response in the germinal centers, variable regions of the Ig heavy chain genes (V_H) are modified by somatic mutations, followed by affinity selection of the cells through antigen binding. Previous studies showed that somatic mutations are introduced into rearranged V_H genes in the course of T cell-dependent immune reactions.[8, 9)] There are three framework regions (FR) and two complementarity-determining regions (CDR) in the V_H gene. Jacob *et al.*[8)] reported that antigen-selected sequences tend to accumulate more mutations in the CDR than in the FR. Antigen-mediated mutation might lead to silent mutations in the FR and replacement mutations in the CDR. This antigen-dependent mutation pattern has been reported in follicle center cell lymphomas,[10)] low-grade MALT lymphomas,[11)] and diffuse large B-cell lymphomas.[12)] MALT lymphomas generally show a relatively low mutation frequency, about 4%, but follicular lymphoma and diffuse large B-cell lymphoma show high mutation frequency (10–12%) due to continuous accumulation of mutations by on-going hypermutation.[13)]

As antigen stimulation might be involved in the pathogenesis of TL, the DNA sequence of V_H was characterized in 13 cases with TL and three with CLTH as a control in the present study.

[3] To whom correspondence and reprint should be requested.
E-mail: aozasa@molpath.med.osaka-u.ac.jp

MATERIALS AND METHODS

Cases Thirteen cases with TL and three with CLTH were selected for the current study: they were admitted with increasing goiter to the Kuma Hospital, Kobe, during the period 1995–98. There were 3 males and 10 females with ages ranging from 45 to 81 (median 68) years in TL. The CLTH patients were females with ages of 45, 56, and 70 years. They received open biopsy or hemithyroidectomy. Histologic specimens were fixed in 10% formalin and routinely processed for paraffin-embedding. Histologic sections, cut at 4 μm, were stained with hematoxylin and eosin and by means of immunoperoxidase procedures. Criteria for the diagnosis of CLTH included increased consistency of the thyroid gland, occasional hypothyrodism, high level of thyroid stimulating hormone, low ^{123}I-uptake, and the presence of antimicrosomal and/or antithyroglobulin antibodies in the serum. Histologic findings of CLTH included lymphocytic infiltration usually forming lymphoid follicles with germinal centers, varying degrees of fibrosis, and oxyphilic change or squamous metaplasia of epithelial cells of the thyroid follicles. Lymphomas were classified according to the revised European-American classification for lymphoid neoplasms (REAL). Immunohistochemical study on the paraffin sections was carried out using the avidin-biotin-peroxidase complex method: monoclonal antibodies used as primary antibody included L26 (CD20), CD3, UCHL-1 (CD45RO) and BCL-2 (DAKO, Glostrup, Denmark), MB-1 and MT-1 (CD43) (Bioscience, Emmenbrucke, Switzerland). All were B-cell lymphomas, and were classified as marginal zone lymphoma (MZL) in 5 cases, follicular center cell lymphoma (FCCL) in 4, and diffuse large B-cell lymphoma (DLBCL) in 4. Four cases of FCC showed a predominantly or partially nodular pattern of proliferation consisting of centroblastic and centrocytic cells, which was distinct from follicular colonization of MZL cells.

Parts of the specimens were snap-frozen at −180°C in liquid nitrogen, and stored at −80°C until use. Genomic DNA was extracted from the frozen specimens by means of the phenol-chloroform method.

PCR amplification of V_H genes Rearranged *Ig* V_H genes were amplified from 100 ng of extracted DNA using six 5′ V_H family-specific leader primers (V_H*1*, 5′-CCTCAGTGAAGTTCCTGCAAGGC-3′; V_H*2*, 5′-GTCCTGCGCTGGTGAAACCACACA-3′; V_H*3*, 5′-GGGGTCCCTGAGACTCTCCTGTGCAG-3′; V_H*4*, 5′-GACCCTGTCCCTCACCTGCCTGTC-3′; V_H*5*, 5′-AAAAAGCCCGGGGAGTCTCTGAGA-3′; V_H*6*, 5′-ACCTGTGCCATCTCCGGGGACAGTG-3′) in combination with either of two 3′ heavy chain joining region primers (J_H*1–5*, 5′-GGTGACCAGGGTCCTGGCCCCAG-3′; and J_H*6*, 5′-GGTGACCGTGGTCCCTTGCCCCCAG-3′) as described by Kupper *et al*.[14] The reaction mixture (50 μl) contained 100 ng of genomic DNA, 0.2 m*M* of the appropriate primer pair, 0.25 m*M* of each deoxynucleotide triphosphate, 1× PCR buffer, and 0.6 U Ampli *Taq* Gold DNA polymerase (Perkin-Elmer Cetus, Norwalk, Connecticut). PCR conditions were as follows: 95°C for 10 min followed by 35 cycles of 95°C for 90 s, 61°C for 30 s, and 72°C for 80 s, and a final elongation at 72°C for 10 min for V_H*1*, *2*, *5* and *6*, and 95°C for 10 min followed by 31 cycles of 95°C for 90 s, 61°C for 30 s, and 72°C for 80 s, and a final elongation at 72°C for 10 min for V_H*3* and *4*.

Cloning and sequencing of PCR products PCR products were electrophoresed on a 1.5% agarose gel, and stained with ethidium bromide. The single band was cut out, purified using QIAquick PCR Purification Kit (Qiagen, Valencia, CA), and cloned in pCR 2.1-TOPO (Invitrogen, Carlsbad, CA). The sequencing reaction was perfomed with the dideoxy chain termination method using an Ampli *Taq* FS Cycle Sequencing Kit (Perkin-Elmer Cetus) according to the manufacturer's protocol. Excess dye terminators were removed by ethanol precipitation. The precipitates were resolved in Template Suppression Reagent and analyzed with the ABI Prism 310 Genetic Analyser (both from Perkin-Elmer Cetus).

Sequence analysis Analysis of sequences was performed using Wisconsin GCG software (Madison, WI) and the GenBank Database. Mutations in the variable region were identified by comparing the consensus sequence of each case with the published germlines. Analysis of somatic mutations of sequences was carried out according to the method described by Bahler *et al*.[15] A binomial mutation model was used to calculate the probability (*P*) that the observed replacement (R) mutations in the CDRs arise by chance.

RESULTS

Usage of V_H family gene and homologous germline gene Genomic DNA isolated from 13 TL tissues and 3 CLTH tissues were analyzed for rearrangement of the *Ig* V_H gene. PCR amplification of rearranged V_H genes was performed with the use of twelve combinations of primers. Eleven of 13 TL tissues (85%) revealed a single band of about 350 bp length. These bands were obtained with only one of twelve combinations of primers, and were cut off, cloned, and sequenced. Ten to 15 clones per case were analyzed. Four (cases 1, 2, 3, and 4) of 11 cases had an identical CDR3 sequence in the major clones. The other 7 had the identical CDR3 sequence in only a few clones, indicating the presence of large numbers of mixed reactive lymphocytes. No bands were observed in the PCR products of the three CLTH cases.

Four TL cases (cases 1, 2, 3, and 4) showed sequences almost homologous with the corresponding germline genes published in the GenBank Database, i. e., *V3-30*, *DP-74*,

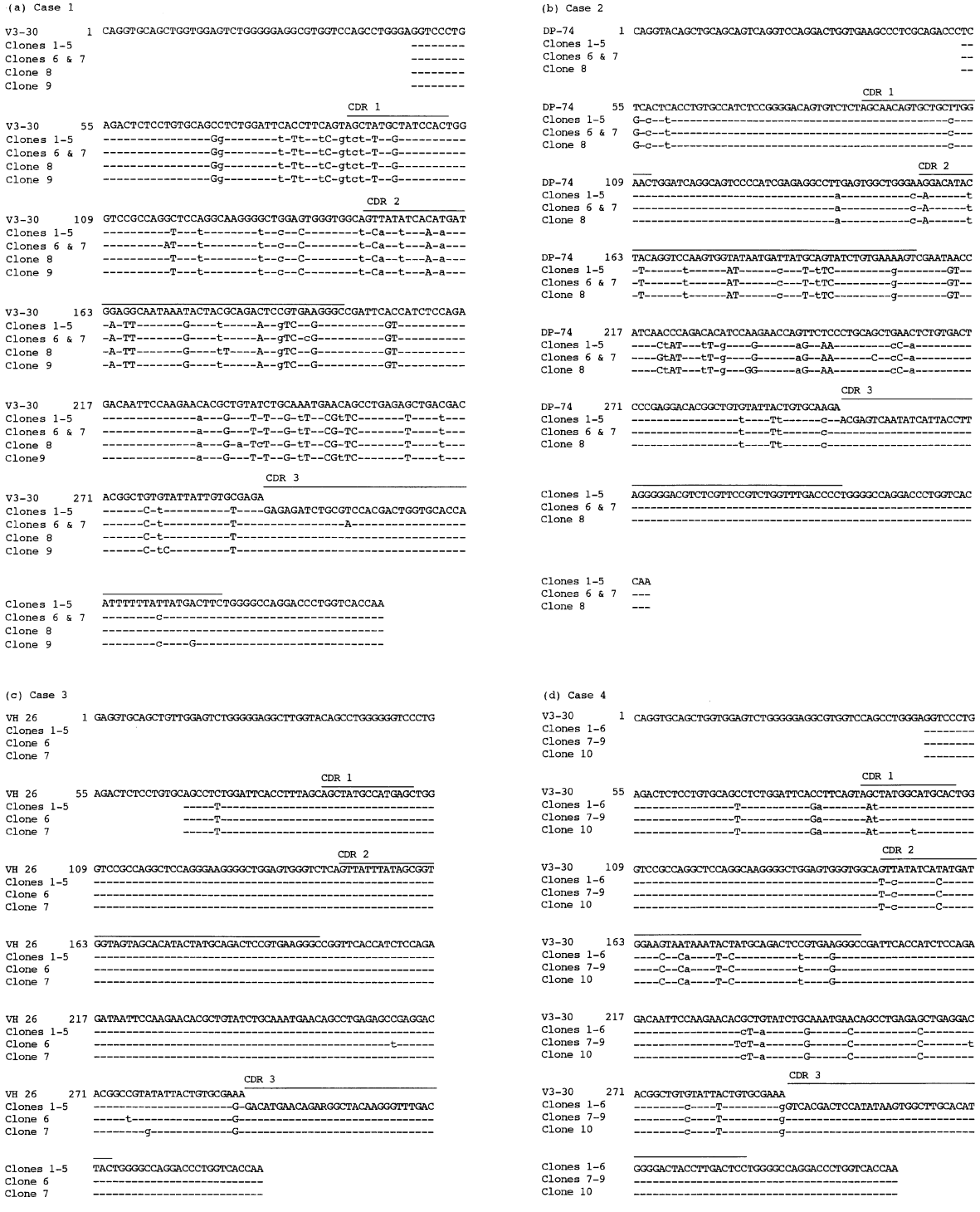

Fig. 1. Cloned nucleotide sequences of *Ig* V_H genes from TL. Comparisons were made with the most homologous germline V_H genes. (a), (b), (c), and (d) represent case1, case 2, case 3, and case 4, respectively. Dashes represent identity with the representative germline sequence. Dots represent excess and/or lack of nucleotide. Capital letters represent replacement mutation, and small letters represent silent mutation. The appropriate nucleotide number and the functional immunoglobulin domains as CDRs are indicated above the sequence as defined by Kabat *et al.*[16]

Table I. Mutation of V_H Gene in Thyroid Lymphoma

Case	Histologic type	Homologous germ line gene	Homology (%)
1	MZL	*V3-30* (V_H3)	79
2	MZL	*DP-74* (V_H6)	84
3	FCCL	*VH26* (V_H3)	99
4	DLBCL	*V3-30* (V_H3)	90

MZL, marginal zone lymphoma; FCCL, follicular center cell lymphoma; DLB, diffuse large B-cell lymphoma.

Table II. Amino Acid Sequence of CDR3 and FR4 in Thyroid Lymphoma

Case	CDR3 segment	FR4 segment
1	ERSATTTGAPIFYYDF	WGQDPGHQ
2	TSQYHYLRGTSRSVWFDP	WGQDPGHQ
3	DMNRWLQGFDY	WGQDPGHQ
4	VTTPYKWLAHGDYLDS	WGQDPGHQ

VH26, and *V3-30*, respectively (Fig. 1 and Table I). V_H3 family gene was used in three TL cases (cases 1, 3, and 4), but the predicted amino acid sequences of CDR3 were different among these cases (Table II). Abnormal sequences which result in frameshift or a stop codon were not observed, indicating that these genes were functional.

Mutation analysis of V_H gene Homology of V_H gene in 4 TL cases ranged from 79 to 99% (average 12%) (Table I). Nucleotide differences between each case and the corresponding homologous germline are shown in Fig. 1 and summarized in Table III. Although the mutation frequency was different among cases, the observed frequencies were higher than expected in the CDRs and vice versa in FRs, except for case 3. The ratios of replacement:silent (R/S) mutation were larger in CDRs than in FRs in these three cases. Case 3 had only two mutations, one in FR1 and the other in FR3. As R/S values are influenced by the numbers of R and S mutations, the probability (P) of R mutation in CDRs was calculated according to the method described by Bahler *et al.*[14] The P values obtained from four TL cases ranged from 0.008 to 0.593.

On-going mutation V_H segment in all four cases showed intraclonal sequence heterogeneity, indicating on-going somatic mutation (Fig. 1 and Table IV). Case 1 had four minor clones; two R mutations and three S mutations in clones 6 and 7, one R mutation and three S mutations in clone 8, and two R mutations and one S mutation in clone 9. Case 2 had three minor clones; two R mutations in clones 6 and 7, and one R mutation in clone 8. Case 3 had two minor clones; two S mutations in clones 6, and one S mutation in clone 7. Case 4 had four minor clones; one R mutation and one S mutation in clones 7–9, and one S mutation in clone 10. As the frequency of the PCR error used in this study was one in 5000–10 000 nucleotides, the intraclonal sequence heterogeneity observed here might not be derived from PCR errors, but should reflect the existence of minor clones in the tumors.

DISCUSSION

Clonal rearrangement of the *Ig* heavy chain gene was found in 11 (85%) of 13 TL cases; all five of MZL, all four of FCCL, and two of four DLBCL. This frequency was identical to that reported previously for TL.[17] Cloning study with sequencing of the CDR3 region revealed the presence of a major clone in four (two MZL, one FCCL, and one DLBCL) of these cases, but a few minor clones in the remaining seven cases. The presence of a major clone in MALT lymphoma of stomach and salivary gland was reported to be 4 of 9 cases[8] and 15 of 20 cases,[9] respectively. This might be due to the admixture of a large number of reactive B-lymphocytes generated through CLTH.

TL is supposed to arise among active lymphoid cells in CLTH. This might suggest a need for antigen stimulation

Table III. Somatic Mutations of the Rearranged V_H Genes in Thyroid Lymphoma

Case	No. of nucleotide differences Observed/Expected		Type of mutation Replacement:Silent		Probability of replacement mutation in CDRs (P)
	FR1+2+3	CDR1+2	FR1+2+3	CDR1+2	
1	30/39	16/14	16:14 (1.14)	10:6 (1.67)	0.034
2	24/27	11/12	11:13 (0.85)	5:6 (0.83)	0.159
3	2/1	0/1	2:0 (∞)	0:0 (—)	0.593
4	12/18	12/6	7:5 (1.40)	8:4 (2.00)	0.008

Table IV. On-going Mutation in V_H Gene in Thyroid Lymphoma

Case	Frequency of minor clone (%)	Mutation pattern in minor clone (position, changes in codons and amino acids)								
1	44	Clones 6 & 7			Clone 8			Clone 9		
		No.118 (FR2)	GTT→ATT	V→I	No.181 (CDR2)	GCA→TCA	A→S	No.280 (FR3)	TAT→CAT	Y→H
		No.193 (CDR2)	AGG→CGG	R→R	No.237 (FR3)	GTG→GTA	V→V	No.338 (CDR3)	GAC→GGC	D→G
		No.252 (FR3)	CGT→CGC	R→R	No.240 (FR3)	TTT→TTC	F→F			
		No.307 (CDR3)	TCC→ACC	S→T	No.252 (FR3)	CGT→CGC	R→R			
		No.333 (CDR3)	TAT→TAC	Y→Y						
2	38	Clones 6 & 7			Clone 8					
		No.221 (FR3)	ACT→AGT	T→S	No.235 (FR3)	AGG→GGG	R→G			
		No.255 (CDR2)	CAG→CAC	Q→H						
3	29	Clone 6			Clone 7					
		No.221 (FR3)	GCC→GCT	A→A	No.279 (FR3)	GTA→GTG	V→V			
		No.276 (FR3)	GCC→GCT	A→A						
4	40	Clones 7–9			Clone 8					
		No.233 (FR3)	ACC→ATC	T→I	No.99 (CDR1)	GGC→GGT	G→G			
		No.270 (FR3)	GAC→GAT	D→D						

for the development of TL. Tumor growth seems to proceed in gastric mucosa-associated lymphoid tissue (MALT) lymphoma that develops regularly in association with *Helicobacter pylori* and requires tumor-infiltrating T-cells for growth by antigen triggering.[18, 19] These gastric MALT lymphomas showed biased usage of V_H germline genes of DP-54 and DP-47.[20] Biased usage of V_H germline genes of *V1-69* was reported in the MALT lymphomas of salivary gland.[21] From the viewpoint of the cellular origin of TL among lymphoid cells in CLTH, the TL might share a common usage of V_H gene. McIntosh *et al.* reported a biased usage of V_H3 and V_H4 genes with the homologous germline of *VH26* in V_H3 gene in B-lymphocytes from CLTH lesions.[22, 23] Three of four TL in the current series used V_H3 gene with the homologous germline of *V3-30* in two cases and *VH26* in one case. Although V_H3 family gene is in general most frequently used not only in B-cell lymphomas, but also in normal peripheral blood lymphocytes, the probability of 0.01 for usage of *VH26* suggested development of TL among active lymphoid cells in CLTH. Though *V3-30* has not been reported from CLTH, the probability of 0.0012 for usage of *V3-30* in two of four cases indicates biased usage of V_H in TL.

Compared to the homologous germline, the mutation frequency of TL ranged from 1 to 21% with an average of 12%, which is comparable with that in gastric MALT lymphoma.[20] A case of follicular center cell lymphoma (case 3) showed only two mutations from the FR1 to FR3 regions. Although somatic mutations are known to accumulate in V_H gene in follicular lymphoma,[24–26] minor nucleotide difference from the germline gene has been reported.[27] Both mutations in case 3 yield amino acid replacement, indicating on-going mutation.

Non-random distribution of replacement and silent mutations within V_H gene segments found in our cases is reported to indicate that these V_H genes are selected by antigen.[8] A review of the literature revealed only one case of TL in which sequences of V_H gene had been analyzed[23] : it was reported that the corresponding homologous germline gene was *V1-03* with 88.8% homology and the probability of R mutation in CDRs was not significantly low ($P>0.05$).[28] However, two cases (case 1 and 4) in our series had a statistically significantly low P value ($P=0.034$ and 0.008, respectively) for R mutation in CDRs. These findings suggest that antigen selectivity through the development of TL works in some cases of TL. The intraclonal mutations were most frequent in FR3. Nine of 20 mutations were replacement mutations, which indicates antigen-selected expansion of proliferating B-lymphocytes.

As shown in Fig. 1 and Table IV, all four cases with TL showed intraclonal heterogeneity in V_H gene: the frequency of the occurrence of minor clones ranged from 29 to 44% per case. These findings indicate the presence of on-going mutation in cases of TL, i.e., the TL arise at the differentiation stage of germinal center B-cells, as revealed in gastric MALT lymphomas[20, 21] and salivary gland MALT lymphomas.[21] Taken together, DNA sequencing of immunoglobulin V_H gene suggests that TL develops among activated lymphoid cells in CLTH at the germinal center stage under antigen selection.

ACKNOWLEDGMENTS

We thank Mr. Youichi Tani (Dako Japan) for immunohistochemical analysis of cases. This study was supported in part by grants (10042005, 10151225, 11470353, 11670212, 11680546, 12576004, 12770087) from the Ministry of Education, Science, Sports and Culture, Japan.

(Received May 7, 2001/Revised July 12, 2001/Accepted July 18, 2001)

REFERENCES

1) Aozasa, K., Ueda, T., Katagiri, S., Matsuzaka, F., Kuma, K. and Yonezawa, T. Immunologic and immunohistologic analysis of 27 cases with thyroid lymphomas. *Cancer*, **60**, 969–973 (1987).
2) Freeman, C., Berg, J. W. and Cutler, S. J. Occurrence and prognosis of extranodal lymphomas. *Cancer*, **29**, 252–260 (1972).
3) Aozasa, K., Tsujimoto, M., Sakurai, M., Honda, M., Yamashita, K., Hanada, M. and Sugimoto, A. Non-Hodgkin's lymphomas in Osaka, Japan. *Eur. J. Cancer Clin. Oncol.*, **21**, 487–492 (1985).
4) Volpe, R. Thyroiditis: current views of pathogenesis. *Med. Clin. North Am.*, **59**, 1163–1175 (1986).
5) Holm, F. M., Blomgren, H. and Lowhagen, H. Cancer risks in patients with chronic lymphocytic thyroiditis. *N. Engl. J. Med.*, **312**, 601–604 (1985).
6) Kato, I., Tajima, K., Suchi, T., Aozasa, K., Matsuzuka, F., Kuma, K. and Tominaga, S. Chronic thyroiditis as a risk factor of B-cell lymphoma in the thyroid gland. *Jpn. J. Cancer Res.*, **76**, 1085–1090 (1985).
7) Dyan, C. M. and Daniels, G. H. Chronic autoimmune thyroiditis. *N. Engl. J. Med.*, **335**, 99 (1996).
8) Jacob, J., Przylepa, J., Miller, C. and Kelsoe, G. *In situ* studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl: III—the kinetics of V-region mutation and selection in germinal center B cells. *J. Exp. Med.*, **178**, 1293–1307 (1993).
9) Berk, C., Berger, A. and Apel, M. Maturation of the immune response in germinal centers. *Cell*, **67**, 1121–1129 (1991).
10) Zelenetz, A. D., Chen, T. T. and Levy, R. Clonal expansion in follicular lymphoma occurs subsequent to antigen selection. *J. Exp. Med.*, **176**, 1137–1148 (1992).
11) Qin, Y., Greiner, A., Hallas, C., Haedicke, W. and Muller-Hermelink, H. K. Intraclonal offspring of gastric MALT-type lymphoma: evidence for the role of antigen-driven high-affinity mutation in lymphomagenesis. *Lab. Invest.*, **86**, 477–485 (1997).
12) Hsu, F. J. and Levy, R. Preferential use of VH4 Ig gene family by diffuse large-cell lymphoma. *Blood*, **86**, 3072–3082 (1995).
13) Matolcsy, A., Nador, R. G., Cesarman, E. and Knowles, D. M. Immunoglobulin JH gene mutational analysis suggests that primary effusion lymphomas derive from different stages of B cell maturation. *Am. J. Pathol.*, **153**, 1609–1614 (1998).
14) Kuppers, R., Zhao, M., Rajewsky, K. and Hansmann, M. L. Detection of clonal B cell populations in paraffin-embedded tissues by polymerase chain reaction. *Am. J. Pathol.*, **143**, 230–239 (1993).
15) Bahler, D. W. and Levy, R. Clonal evolution of a follicular lymphoma: evidence for antigen selection. *Proc. Natl. Acad. Sci. USA*, **89**, 6770–6774 (1992).
16) Kabat, E. A., Wu, T. T., Perry, H. M., Gottesman, K. S. and Foeller, C. "Sequences of Proteins of Immunological Interest," 5th Ed. (1991). US Department of Health and Human Services, NIH Publication, Bethesda, MD.
17) Matsuzuka, F., Fukata, S., Kuma, K., Miyauchi, A., Kakudo, K. and Sugawara, M. Gene rearrangement of immunoglobulin as a marker of thyroid lymphoma. *World J. Surg.*, **22**, 558–561 (1998).
18) Hussell, T., Issacson, P. G., Crabtree, J. E. and Spencer, J. *Helicobacter pylori*-specific tumor infiltrating T cells provide contact dependent help for the growth of malignant B cells in low-grade gastric lymphoma of mucosa-associated lymphoid tissue. *J. Pathol.*, **178**, 122–127 (1996).
19) Hussell, T., Issacson, P. G., Crabtree, J. E., Dogan, A. and Spencer, J. Immunoglobulin specificity of low grade B cell lymphoma of mucosa-associated lymphoid tissue (MALT) type. *Am. J. Pathol.*, **142**, 285–292 (1993).
20) Hallas, C., Greiner, A., Peters, K. and Muller-Hermelink, H. K. Immunoglobulin VH genes of high-grade mucosa-associated lymphoid tissue lymphomas show high load of somatic mutations, and evidence of antigen-dependent affinity maturation. *Lab. Invest.*, **78**, 277–287 (1998).
21) Miklos, J. A., Swerdlow, S. H. and Bahler, D. W. Salivary gland mucosa-associated lymphoid tissue lymphoma immunoglobulin VH genes show frequent use of V1-69 with distinctive CDR 3 features. *Blood*, **95**, 3878–3884 (2000).
22) McIntosh, R. S., Tandon, N., Metcalfe, R. A. and Weetman, A. P. Cloning and analysis of IgM anti-thyroglobulin autoantibodies from patients with Hashimoto's thyroiditis. *Biochim. Biophys. Acta*, **1227**, 171–176 (1994).
23) McIntosh, R. S., Asghar, M. S., Watson, P. F., Kemp, E. H. and Weetman, A. P. Cloning and analysis of IgGκ and IgGλ anti-thyroglobulin autoantibodies from a patient with Hashimoto's thyroiditis. Evidence for *in vivo* antigen-driven repertoire selection. *J. Immunol.*, **157**, 927–935 (1996).
24) Kon, S., Levy, S. and Levy, R. Retention of an idiotypic determinant in a human B-cell lymphoma undergoing immunoglobulin variable-region mutation. *Proc. Natl. Acad. Sci. USA*, **84**, 5053–5057 (1987).
25) Cleary, M. L., Meeker, T. C., Levy, S., Lee, E., Trela, M., Sklar, J. and Levy, R. Clustering of extensive somatic mutations in the variable region of an immunoglobulin heavy chain gene from a human B cell lymphoma. *Cell*,

44, 97−103 (1986).
26) Cleary, M. L., Galili, N., Trela, M., Levy, R. and Sklar, J. Single cell origin of bigenotypic and biphenotype B cell proliferations in human follicular lymphomas. *J. Exp. Med.*, **167**, 582−597 (1988).
27) Bahler, D. B., Campbell, M. J., Hart, S., Miller, R. A., Levy, S. and Levy, R. Ig V_H gene expression among human follicular lymphomas. *Blood*, **78**, 1561−1568 (1991).
28) Lossos, I. S., Okada, C. V., Tibshirani, R., Warnke, R., Vose, J. M. and Levy, R. Molecular analysis of immunoglobulin genes in diffuse large B-cell lymphomas. *Blood*, **95**, 1797−1803 (2000).
29) Du, M., Diss, T. C., Peng, H., Issacson, P. G. and Pan, L. Ongoing mutation in MALT lymphoma immunoglobulin gene suggests that antigen stimulation plays a role in the clonal expansion. *Leukemia*, **10**, 1190−1197 (1996).