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Most of CD30$^+$ anaplastic large cell lymphoma of B cell type show a somatic mutation in the IgH V region genes

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Relationship and histogenesis of Hodgkin's disease (HD) and anaplastic large cell lymphoma (ALCL) still remain unclear. Recently, Reed–Sternberg cells or Hodgkin cells in HD with B cell phenotype (B-HD) are considered to originate from germinal center B cells, ALCLs of B cell phenotype (B-ALCL) are involved in diffuse large B cell lymphoma (DLBCL) as anaplastic variant, but an origin of tumor cells of B-ALCL has not been elucidated. We have therefore investigated somatic mutation of the Ig heavy chain (IgH) genes among 17 cases of B-ALCL to clarify whether there is a difference in characteristic and origin of tumor cells between B-ALCL, B-HD and DLBCL. Amplificates of IgH variable (V) region of 10 cases by the polymerase chain reaction method were sequenced and compared with reported germ line configurations. Nine cases (90%) with heavily somatic mutations were found. A case with an out-of-frame rearrangement and a case with 9 base pairs insertion were included. The mutation pattern revealed the tumor cells were selected for antibody expression and discriminated from B-HD. These findings suggest the tumor cells of B-ALCL are derived from germinal center or postgerminal center (memory and effector) B cells and an origin of B-ALCL is not different from DLBCL.

Keywords: anaplastic large cell lymphoma; B cell; Ig heavy chain; somatic mutation

Introduction

Ki-1 (CD30) was first reported as a specific antigen for Hodgkin and Reed–Sternberg (HRS) cells of Hodgkin's disease (HD)[1] and has subsequently reported to be expressed in some non-Hodgkin's lymphomas including anaplastic large cell lymphoma (ALCL). ALCL is recognized as a new entity of non-Hodgkin's lymphoma.[2,3] Although histological findings such as the presence of a pure sinusoidal growth pattern and the absence of diagnostic HRS cells, or immunohistological findings such as CD45$^+$ and CD15$^-$ can be helpful in the diagnosis of ALCL, the distinction between ALCL and HD is not easy[4–6] and the histogenesis of ALCL and HD still remains unclear.

Somatic mutation of rearranged Ig heavy chain (IgH) variable (V) region genes of B cells occurs during their differentiation in the germinal centers and plays a major role in generating antibody diversity.[7–10] The tumor cells without somatic mutation of rearranged IgH V region genes are derived from pregerminal (naive) B cells, whereas the tumor cells with somatic mutation of rearranged IgH V region genes are derived from germinal center or postgerminal center (memory and effector) B cells.[10,11] The analysis of the sequence of rearranged IgH V region genes has proved to be suitable for determining the stage of differentiation of normal and tumor B cells. Somatic mutation of rearranged IgH V region genes have been found in follicular lymphoma,[12–14] Burkitt's lymphoma,[14] multiple myeloma,[15] mucosa-associated lymphoid tissue (MALT)-type lymphoma[16] and diffuse large B-cell lymphoma (DLBCL),[17,18] but not in mantle cell lymphoma (MCL)[19] or B-acute lymphocytic leukemia.[20] B-chronic lymphocytic leukemia (B-CLL) is reported to comprise cases with germline[21–23] and cases with somatic mutation.[24] HD with B cell phenotype (B-HD) showed somatic mutation of rearranged IgH V region genes or not, suggesting the HRS cells originate from B-lineage cells at various stages of development.[25–27] Recently, however, B-HD of both the lymphocyte predominance (LP) and classical types has been recognized to carry clonal rearrangements of the Ig gene with somatic mutation; HRS cells of B-HD were derived from germinal center cells.[28–31] Somatic mutation analysis of rearranged IgH V region genes has not yet been applied in ALCL with B cell phenotype (B-ALCL), except in only one case.[18]

We have recently investigated the clinical features, immunophenotypes, and Epstein–Barr virus (EBV) gene expression in 17 cases of B-ALCL and provided evidence of a possible association of EBV with B-ALCL, particularly large pleomorphic cells.[32] In this study, we have analyzed the sequence of rearranged IgH V region genes using extracted DNA from 10 cases of B-ALCL, in order to elucidate whether there is a difference in characteristic and origin of tumor cells between B-ALCL, B-HD and DLBCL. The results have revealed that most of the B-ALCL cases show mutated IgH V region genes, indicating that tumor cells of B-ALCL are derived from germinal center or postgerminal center (effector or memory) B cells.

Materials and methods

Cases

Seventeen cases with B-ALCL filed in the First Department of Pathology, Fukushima Medical College were used for the present study. A diagnosis of B-ALCL was established according to the histopathological criteria of Stein *et al*[4] and Agnarsson and Kadin,[5] as well as the presence of CD30 and CD20 reactivity. The series consisted of 10 cases of monomorphic type and seven cases of pleomorphic type. The clinicopathological features, immunophenotypes, and EBV gene expression have been previously described.[32] The summary is shown in Table 1.

PCR amplification and sequence analysis

DNA samples obtained from frozen tissue of 17 cases with B-ALCL were digested with proteinase K, extracted with phenol/chloroform and precipitated by ethanol.

A semi-nested polymerase chain reaction (PCR) was performed using DNA Thermal Cycler (Perkin-Elmer Cetus, Nor-

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Table 1 Clinical features, immunophenotype, and EBV gene expression in 17 cases of B-ALCL

Case	Age/Sex	Site[a]	Prognosis (months)	CD20	CD30	CD45	EBER1[b]	LMP[c]	EBNA2[c]
Monomorphic type									
1	57/M	*Mediastinum*	69, alive	+	+	+	−	−	−
2	82/F	*Subcutis*, Cervical LN	12, dead	+	+	+	−	−	−
3	78/M	*Axillary LN*, Inguinal LN	19, alive	+	+	+	+	+	−
4	42/F	*Cervical LN*, Axillary LN, Splenomegaly, Hepatic hilar LN	6, dead	+	+	+	−	−	−
5	35/F	*Cervical LN*	30, alive	+	+	+	−	−	−
6	45/M	*Cervical LN*	2, dead	+	+	+	−	−	−
7	82/F	*Supraclavicular LN* Cervical LN, Axillary LN	13, dead	+	+	+	+	+	−
8	41/F	*Uterus*	4, alive	+	+	+	−	−	−
9	71/F	*LN*	3, dead	+	+	+	−	−	−
10	54/F	*Cervical LN*, Inguinal LN, Paraaortic LN	13, alive	+	+	+	−	ND	ND
Pleomorphic type									
11	61/M	*Axillary LN*	73, alive	+	+	+	+	+	+
12	81/M	*Parotid gland*, Cervical LN	5, dead	+	+	+	−	−	−
13	48/M	*Cervical LN*, Paraaortic LN Liver SOL, Splenomegaly	1, dead	+	+	+	−	−	−
14	63/M	*Cervical LN*	3, dead	+	+	+	+	+	−
15	63/M	*Supraclavicular LN*	26, alive	+	+	+	+	+	+
16	69/F	*Cervical LN*	15, alive	+	+	+	−	−	−
17	54/M	*Cervical LN*	1, alive	+	+	+	+	+	−

[a]The biopsy material is in italics.
[b]EBER1 was detected by RNA *in situ* hybridization.
[c]LMP1 and EBNA2 were detected by immunohistochemistry.
ND, not done.

walk, CT, USA), according to a previously described method.[25,33] In brief, the first amplification was performed using an upstream consensus V region primer (FR2A) and a lowstream joining (J) region primer (LJH). For the reamplification, the LJH was replaced by a nested consensus J region primer (VLJH), and an amplified product (1%) of the first round was transferred as a template. Ten microliters of each amplification product were separated by electrophoresis on a 6% polyacrylamide gel and visualized by staining with ethidium bromide.

The DNA sequence analysis was performed by the dideoxy-chain termination method, using the Sequenase PCR Product Sequencing Kit (USB, Ohio, USA). By application of FR2A and VLJH primers, respectively, a determination of the DNA sequence of the upper and lower strands was established. Only those cases completely homologous between both sequences were chosen for comparison with published V region germ line sequences, using the BLASTN and the GenBank database.

Results

The IgH gene rearrangement was detected by the PCR method in 10 of 17 (59%) cases with B-ALCL, comprising six of 10 cases (60%) with monomorphic type and four of seven cases (57%) with pleomorphic type. The amplificates obtained were sequenced and compared with published germline V region sequence (Table 2). The other seven cases failed to be sequenced because three cases showed no PCR product and four cases showed multiple bands on backgrounds. Among 10 cases examined, nine (90%) showed somatic mutation of rearranged IgH V region genes. A case (case 11) had an out-of-frame rearrangement caused by one base deletion and another case (case 15) had an in-frame rearrangement with nine bases insertion. Both were of pleomorphic type. Only one case (case 6) with monomorphic type showed a germline sequence of IgH V region gene. The average of mutation frequency of IgH V region in nine cases with somatic mutation was 13.0% (range 6.1%–23.1%). The rate of replacement mutation *vs* silent mutation (R/S) in seven cases with the in-frame rearrangement was 1.4, comprising 46 *vs* 18 (2.6) in complementarity determining region II (CDRII) and 39 *vs* 43 (0.9) in framework region III (FWIII) (as shown in Table 3).

Discussion

In the Revised European–American classification of lymphoid neoplasms (REAL),[3] ALCLs of T cell and null-cell type are recognized as an entity, whereas B-ALCLs are involved in DLBCL as an anaplastic variant. They have mentioned that many of ALCLs in children with T cell or null-cell types have shown the (2;5)(p23;q35) translocation, but B-ALCLs do not have such a translocation or any significant difference from other variants of DLBCL in clinical course. Seventeen cases of B-ALCL in our series, however, were somewhat different. We showed that 37% of B-ALCL cases harboured the EBV genome,[32] prognosis of B-ALCLs was better than that of DLBCL and rather similar to that of HD (unpublished data). Further studies, therefore, will be needed.

A semi-nested PCR method for the rearranged IgH gene allowed the detection of around 50 identically rearranged B cells in a mixture of 100 000 nonrearranged or individually rearranged cells.[25] The PCR of IgH gene has been reported to detect rearrangements in 16 of 24 cases (67%) of B-HD, six of 10 cases (60%) or 19 of 28 cases (68%) of DLBCL and six of 12 cases (50%) of follicular center lymphoma (FCL).[18,25]

Table 2 Sequence comparison of the amplificates of B-ALCL with germ line sequences

DP-66	[CDR II]	TATATCTAT TAC AGTGGGAGCACCAACTACAACCCCTCCCTCAAGAGT
Case No. 4		A— —a –C–G— –Cc — — — — — — — — — — —
	[FW III]	CGAGTCACC ATA TCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCTGCGGACACGGCCGTGTATTACTGTGCGAGA
		— — — — —g — — — — — — A— C— — —c —a — –A– — — –A– A— — — — — — — —t — — —
DP-38	[CDR II]	CGTATTAAA AGC AAAACTGATGGTGGGACAACAGACTACGCTGCACCCGTGAAAGGC
Case No. 6		— —
	[FW III]	AGATTCACC ATC TCAAGAGATGATTCAAAAAACACGCTGTATCTGCAAATGAACAGCCTGAAAACCGAGGACACAGCCGTGTATTACTGTACCACA
		— —
V3-64	[CDR II]	GCTATTAGT AGT AATGGGGGTAGCACATATTATGCAAACTCTGTGAAGGGC
Case No. 7		— —c –A– G— —G — –A– —G –G– —c —c —g G— –Ac — — –A–
	[FW III]	AGATTCACC ATC TCCAGAGACAATTCCAAGAACACGCTGTATCTTCAAATGGGCAGCCTGAGAGCTGAGGACATGGCTGTGTATTACTGTGCGAGA
		— — GT– –Ct — — — — —g G— — —t — — — — — AC– –Tt T— — — —C — TC– — TAt — –Tt — — —
DP-63	[CDR II]	GAAATCAAT CAT AGTGGAAGCACCAACTACAACCCGTCCCTCAAGAGT
Case No. 8		— —t –G– — –C– —g –A– — —A — —t — — — — —
	[FW III]	CGAGTCACC ATA TCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGA
		— — –T– —G — —t — G— — — —t — —t — — —a —c At– — —a — –T– — —t — — —t — — — —
DP-46	[CDR II]	GTTATATCA TAT GATGGAAGCAATAAATACTACGCAGACTCCGTGAAGGGC
Case No. 9		–C– — AGg AG– –G– — — — — — — — — — — — — —
	[FW III]	CGATTCACC ATC TCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGA
		— —c — — — — –T–
DP-47	[CDR II]	GCTATTAGT GGT AGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGGC
Case No. 10		TG– — — –A– –AA — — –A– –T– –T– — — — — A— — —
	[FW III]	CGGTTCACC ATC TCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAA
		— A— — — — — — — — — — —a —a — — — — —G — — — –T– — — — — — — — — — –Cg
YAC-9	[CDR II]	CGTATTAGA AGC AAAGCTAACAGTTACGCGACAGCATATGCTGCGTCGGTGAAAGGC
Case No. 11		— —c — GA– — — — –A– — — — — — CT– — — — — —
	[FW III]	AGGTTCACC ATC TCCAGAGATGATTCAAAGAACACGGCGTATCTGCAAATGAACAGCCTGAAAACCGAGGACACGGCCGTGTATTACTGTACTAGA
		— — — — — — — — — — — — —a — — — –*— — — — — — — — — — — — — — —T CC
		one base deletion
DP-67	[CDR II]	AGTATCTAT CAT AGTGGGAGCACCTACTACAACCCGTCCCTCAAGAGT
Case No. 12		–TG —t — — — — –C– G— —t — TGt GAt — — G— —
	[FW III]	CGAGTCACC ATA TCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCCGCAGACACGGCCGTGTATTACTGTGCGAGA
		— — — —t —g A–t — — —t —a —t —a —t —t — — G— –Ct —a A— — —a —t — — — ACt — — — — —
DP-66	[CDR II]	TATATCTAT TAC AGTGGGAGCACCAACTACAACCCCTCCCTCAAGAGT
Case No. 13		— — –T– A–t — AAa —t C— — — — — — — — — —
	[FW III]	CGAGTCACC ATA TCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCTGCGGACACGGCCGTGTATTACTGTGCGAGA
		— — — —c — —g — — — — –CG — — — — — —t –C– — — –T– —c — — — — —c — —t — — —
DP-47	[CDR II]	GCTATTAGT GGT AGTGGTGGTAGCACA *** *** *** TACTACGCAGACTCCGTGAAGGGC
Case No. 15		–T– — TA– –CcG — — TC– –C– — TTC TATGCA— —t — — —t — — —
		9 bases insertion
	[FW III]	CGGTTCACC ATC TCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAA
		— — — — — — —t — — — –G– — — –T– — — — — — — — — — — — — —c — — — — —

Sequence analysis of PCR amplificates. The first line displays the most homologous published IgH V region germline genes when compared with DNA sequences of our PCR products (second line).[38–40] Identical bases are indicated by hyphens. Bases substitution were either written in upper case letters for resulting amino acid (replacement mutation) or in lower case letters without consequences for the resulting amino acid sequence (silent mutation).
CDR, complementarity determining region: FW, framework region.

The PCR method was not always able to detect rearrangements of the IgH gene. We detected distinct IgH rearrangements in 10 of 17 (59%) cases with B-ALCL. No PCR products in three cases were obtained, due to degradation of the DNA or somatic mutations at sites complementary to the primers and the primers lose their binding capacity.[34] Four cases gave rise to multiple PCR products, indicating that those specimens harbor a relatively high fraction of polyclonal non-malignant B cells that prevented identification of the tumor clone. A frequency of detectable IgH gene rearrangements of B-ALCL was similar to that of B-HD, DLBCL and FCL.

Many studies about somatic mutation of Ig V region genes have been reported. Tamaru *et al*[25] reported that six of 10 cases with HD underwent somatic mutation of IgH V region genes, comprising three of four cases with LP-HD and three of six cases with classical HD. Kuppers *et al*[26] demonstrated nonfunctional V region gene rearrangement in one of three cases with HD by the sequence analysis of micromanipulated HRS cells. Hummel *et al*[27] reported that HRS cells carrying polyclonal Ig gene rearrangement in a large fraction of cases might exist. Those data suggested that HRS cells of B-HD were heterogeneous in B cell differentiation. Recent reports, however, indicate that both LP and classical HD carry clonal rearrangements of Ig gene with somatic mutation.[28–31] In DLBCL, most, if not all cases, showed somatic mutation of Ig V region genes.[17,18] Kuppers *et al*[18] reported 19 cases of DLBCL consisting of 10 cases of centroblastic lymphoma, five cases of mediastinal B cell lymphoma, two cases of immunoblastic lymphoma, one case T cell-rich B cell lymphoma and one case of large cell anaplastic lymphoma were harbored mutated Ig V region genes and this finding lent support to the concept of the REAL classification to group those lymphomas as an entity. In our series, B-ALCL showed replacement and silent mutation of IgH V region genes in nine of 10 cases (90%). Amplified rearrangements of four cases of pleomorphic type were detected in somatic mutation of IgH V region. These findings suggest that the tumor cells of B-ALCL are derived from germinal center or postgerminal center (effector or memory) B cells in B cell differentiation. The average of mutation frequency of IgH V region genes in B-ALCL was 13.0% (range 6.1%–23.1%). Averages of mutation frequency of IgH V region genes in various types of B cell lymphoma were reported; 8.5% and 11% in DLBCLs,[17,18] 13.2% and 10.7% in B-HD,[25,29] 11.8% in FCL,[14] 4.9% in sporadic

Table 3 Distribution of replacement and silent mutation in rearranged IgH V region genes of B-ALCL

Case	Germline usage[a]	Region	Base	Replacement	Silent	Total
Monomorphic type						
4	VH4/DP-66	CDR II	48	4 (8.3)	2 (4.2)	6 (12.5)
		FW III	96	5 (5.1)	4 (4.1)	9 (9.1)
6	VH3/DP-38	CDR II	57	0 (0.0)	0 (0.0)	0 (0.0)
		FW III	96	0 (0.0)	0 (0.0)	0 (0.0)
7	VH3/V3-64	CDR II	51	9 (17.6)	5 (9.8)	14 (27.5)
		FW III	96	14 (14.6)	6 (6.3)	20 (20.8)
8	VH4/DP-63	CDR II	48	4 (8.3)	3 (6.3)	7 (14.6)
		FW III	96	5 (5.2)	9 (9.4)	14 (14.6)
9	VH3/DP-46	CDR II	51	6 (11.8)	1 (2.0)	7 (13.7)
		FW III	96	1 (1.0)	1 (1.0)	2 (2.1)
10	VH3/DP-47	CDR II	51	9 (17.6)	0 (0.0)	9 (17.6)
		FW III	96	4 (4.2)	3 (3.1)	7 (7.3)
Pleomorphic type						
11	VH3/YAC-9	CDR II	57	5 (8.8)	1 (1.8)	6 (10.5)
		FW III	96	3 (3.1);1D	1 (1.0)	4 (4.2)
12	VH4/DP-67	CDR II	48	9 (18.8)	4 (8.3)	13 (27.1)
		FW III	96	6 (6.3)	14 (14.6)	20 (20.8)
13	VH4/DP-66	CDR II	48	5 (10.4)	3 (6.3)	8 (16.7)
		FW III	96	4 (4.1)	6 (6.3)	10 (10.4)
15	VH3/DP-47	CDR II	51	8 (15.7);9I	3 (5.9)	11 (17.6)
		FW III	96	2 (2.1)	2 (2.1)	4 (4.2)

[a]According to GenBank, using BLASTN for data bank comparison.[38–40]
Case 11 contained one base deletion.
Case 15 contained 9-bases insertion.
The figures presented in parenthesis indicate the percentage.
CDR, complementarily determining region; FW, framework region; D, deletion; I, insertion.

Burkitt's lymphoma,[14] and 5.5% in MALT-type lymphoma.[16] Mutation frequency in B-ALCL was similar to those of B-HD, DLBCL and FCL, but different from those of Burkitt's and MALT-type lymphoma. These data indicate that B-ALCL has heavily mutated IgH V region genes.

The R/S values within Ig V region genes can be taken as an indication for a possible selection of antibody molecule for high-affinity binding. The R/S values in the FW region are usually lower than expected assuming random mutagenesis of the Ig V region genes in B cells selected for antibody expression.[18,35] Kuppers *et al*[18] reported that the R/S value in FW of DLBCLs with in-frame rearrangement was clearly smaller than the calculated value: 1.2 compared to 3.1. This R/S value was in the same range as normal IgM memory B cells (1.5) and class-switched memory B cells (1.0). The R/S value of our seven cases with the in-frame rearrangement was 0.9 in FWIII, which was similar to that reported of DLBCL, normal IgM and class-switched memory B cells. The tumor cell of B-ALCL was also selected for antibody expression. In the reported HD cases, stop codons were found in several in-frame rearrangement and the R/S value in FW was 1.9, indicating that R mutations were not stringently counterselected and the origin of HRS cells resided inside the germinal center.[18,29] In our B-ALCL series, stop codon was not found in cases with in-frame rearrangement, suggesting that the mutation pattern of B-ALCL is different from B-HD.

Molecular analysis of somatic mutation of normal B cells revealed that an out-of-frame rearrangement rarely occurred.[11] In lymphoid malignancies, most reported cases displayed an in-frame rearrangement except a case of DLBCL and some cases of B-HD.[18,25–29,31] In the present study, one case (case 11) of pleomorphic type with an out-of-frame rearrangement deleted one base (T) of 17th codon of FWIII, with the result that the new stop codon (TGA) was formed three codons after that deletion. We found another case (case 15) showing 9-base insertion between the 9th and 10th codon of CDRII without out-of-frame. Interestingly, EBV genome was detected in tumor cells of these two cases by EBER1 RNA-ISH. The two cases expressed EBV-encoded latent membrane protein 1 (LMP1) and EBV-encoded nuclear antigen 2 (EBNA2) and had a favorable prognosis. Most of the reported EBV+ ALCL cases, with the exception of three cases of human immunodeficiency virus-1 (HIV)+ B-ALCL[36] and two cases of HIV− B-ALCL,[37] were negative for EBNA2.

The only case of monomorphic type showed an absence of IgH V region gene mutation. This case was no different from the other B-ALCLs in clinicopathologic, immunologic or morphologic characteristics. No evidence of blastic transformation from B-CLL or MCL was found in this case. There has been no report of high grade lymphoma with absence of IgH V gene mutation, except one case of DLBCL.[18] It was not well evaluated and further studies will be required about such a case.

In conclusion, analysis of IgH V genes for somatic mutations revealed most B-ALCLs had heavily mutated IgH V region genes, indicating that the tumor cells are derived from the germinal center or postgerminal center (effector or memory) B cells. The mutation pattern indicated the tumor cells of B-ALCL are selected for antigen expression and are different from B-HD.

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