Jpn. J. Cancer Res. 88, 1087–1093, November 1997

# Somatic Hypermutations in the $V_H$ Segment of Immunoglobulin Genes of CD5-positive Diffuse Large B-Cell Lymphomas

Masaaki Kume,[1,4] Ritsuro Suzuki,[1] Yasushi Yatabe,[2] Yoshitoyo Kagami,[2] Ikuo Miura,[4] Akira B. Miura,[4] Yasuo Morishima,[3] Shigeo Nakamura[2] and Masao Seto[1,5]

[1]*Laboratory of Chemotherapy, Aichi Cancer Center Research Institute,* [2]*Department of Pathology and Clinical Laboratories,* [3]*Department of Hematology and Chemotherapy, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya 464 and* [4]*Third Department of Internal Medicine, Akita University School of Medicine, 1-1-1 Hondo, Akita 010*

***De novo* CD5-positive ($CD5^+$) diffuse large B-cell lymphoma (DLBL) has recently been identified as constituting a homogeneous subgroup with distinct clinicopathologic and genotypic characteristics, but its origin remains to be elucidated. Previous studies by sequence analysis of the variable region of the immunoglobulin heavy chain ($V_H$) have shown that $CD5^+$ B-cell malignancies such as mantle cell lymphoma (MCL) and B-cell chronic lymphocytic leukemia (B-CLL) cells represent pre-germinal center (pre-GC) stage B cells in contrast with the post-GC stage of most DLBLs, which show somatic hypermutations in $V_H$ genes. In the present study, we investigated the $V_H$ sequence of *de novo* $CD5^+$ DLBL to clarify whether $CD5^+$ DLBL represents the pre-GC stage, as do other $CD5^+$ B-cell malignancies, or the post-GC stage, as is typical of DLBL. All eight cases (four $CD5^+$ DLBL and four CD5-negative ($CD5^-$) DLBL) examined by us showed somatic hypermutations in the $V_H$ segment and two of the $CD5^-$ DLBL cases showed intra-clonal diversity, suggesting that $CD5^+$ DLBLs were derived from the same maturation stage as $CD5^-$ DLBL, but were distinct from the other indolent $CD5^+$ B-cell lymphomas of B-CLL and MCL. These data suggest that *de novo* $CD5^+$ DLBLs do not merely lie within a continuous spectrum with B-CLL and MCL, but represent a biologically distinct variant within the diagnostic framework of diffuse large B-cell lymphoma.**

Key words: Diffuse lymphoma — Somatic mutation — CD5 — Immunoglobulin — Variable region

CD5 is a 67 kDa T cell-associated antigen, but is also expressed in approximately 20% of adult human peripheral blood and spleen B cells, 30% of adult lymph node and tonsil B cells, and 40 to 60% of fetal lymph node and spleen B cells.[1)] Some evidence suggests that these B cells are developmentally and functionally distinct from conventional B cells.[2)] On the other hand, CD5-positive ($CD5^+$) B-cell lymphomas are heterogeneous and consist of several disease entities, including chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SCL), mantle cell lymphoma (MCL), and less commonly diffuse large B-cell lymphoma (DLBL), although current morphologic, immunologic, and molecular parameters do not seem to have clearly established their defining characteristics.[3)]

Identification of DLBL is based on a morphologic diagnosis and includes distinct subgroups, the delineating features of which are expected to be clarified by newer immunophenotypic and molecular markers. Recently, it has been suggested by Knowles' group and ours that *de novo* $CD5^+$ DLBLs constitute a homogeneous subcategory.[4,5)] Matolcsy *et al.* reported that *de novo* $CD5^+$ DLBL may be distinct from DLBLs with Richter's syndrome, because the *bcl-6* gene rearrangement was observed in 44% of their cases, in the absence of structural alterations of *bcl-1*, *bcl-2*, c-*myc*, H-*ras*, K-*ras*, N-*ras* proto-oncogenes and *p53* tumor suppressor genes.[3)] Yatabe *et al.* also indicated that this type of lymphoma appears to be distinct from MCL and nodal CD5-negative ($CD5^-$) DLBLs based on their morphologic and immunohistochemical findings for cyclin D1, bcl-2, p53, and RB proteins.[5)] Interestingly, the patients with the *de novo* $CD5^+$ DLBLs were clinically characterized by female predominance, elderly onset, and an aggressive clinical course almost identical to that of MCL, implying that the immunophenotypic detection of CD5 may help to delineate this distinctive subgroup accounting for 5 to 10% of DLBLs. However, the biologic relationship of these $CD5^+$ DLBLs with the other types of lymphomas, i.e., CLL/SLL, MCL, and $CD5^-$ DLBLs, has not been completely clarified.

An analysis of immunoglobulin heavy chain (IgH) V ($V_H$) genes demonstrated that MCLs and B-CLLs had germline $V_H$ genes and it was suggested that they were derived from pre-germinal center (GC) B cells (naive B cells).[6,7)] In contrast, DLBLs were shown to have a high frequency of $V_H$ somatic mutations, suggesting that they are of post-GC origin.[8)] However, it is not clear whether $CD5^+$ DLBLs are of pre-GC or post-GC origin. In the present study, we analyzed the somatic mutation of the

[5] To whom correspondence should be addressed.

$V_H$ gene in $CD5^+$ DLBLs in order to clarify the latter's origin and demonstrated that their origin is distinct from that of MCLs and CLLs.

## MATERIALS AND METHODS

**Patients and samples** Tumor samples were obtained from 10 cases of $CD5^+$ DLBL, 10 cases of $CD5^-$ DLBL, two cases of MCL and two cases of B-cell CLL (B-CLL). The pathologic diagnoses were established according to the Revised European American Lymphoma (REAL) classification.[9] None of the $CD5^+$ DLBL cases had pre-existing low-grade non-Hodgkin's lymphoma or coexisting chronic lymphocytic leukemia. The immunohistochemistry of PRAD1/cyclin D1 was evaluated and the tumor cell surface markers of each case were also analyzed as described previously.[5, 10] The cases analyzed for sequence are shown in Table I. PRAD1/cyclin D1 was positive for MCL but negative for $CD5^+$ DLBL (data not shown).

**Genomic DNA preparation** Genomic DNA was extracted from the tumor samples as described previously.[11] Briefly, frozen tumor samples were resuspended in 9.5 ml of extraction buffer (10 m*M* Tris-HCl pH 7.4, 10 m*M* EDTA, 150 m*M* NaCl, 0.4% sodium dodecyl sulfate and 500 $\mu$l of 20 mg/ml proteinase K). After overnight digestion at 37°C, the mixture was extracted twice with phenol/chloroform. The aqueous phase was mixed with 2.5 volumes of ethanol and the precipitate washed twice in 70% ethanol. The genomic DNA thus obtained was dried and dissolved in TE buffer (10 m*M* Tris-HCl pH 7.4, 1 m*M* EDTA pH 8.0).

**Polymerase chain reaction (PCR) amplification** A semi-nested PCR was performed as described previously but with a slight modification.[12] Briefly, the first amplification with 100 ng of DNA template was performed by using an upstream consensus $V_H$ primer (FR2A: 400 ng) and a downstream $J_H$ primer (LJH: 100 ng). For the reamplification, an aliquot (1%) of the first PCR was used as a template with a nested consensus primer (VLJH: 200 ng) and the upstream primer (FR2A: 200 ng). Primer annealing consisted of five cycles at 63°C for 60 s, and the remaining 35 cycles at 57°C. The conditions for DNA denaturation (96°C for 30 s) and primer extension (72°C for 60 s) were kept constant for all cycle steps. After the last cycle, synthesis was extended for 10 min. Stringent primer annealing conditions at 63°C for 60 s were kept constant for all cycles of reamplification (25 cycles). Ten microliters of each amplified product was subjected to 2.5% agarose gel electrophoresis and visualized with ethidium bromide.

**DNA sequence analysis** The DNA sequence analysis was performed by means of the dideoxynucleotide chain termination method using the Sequenase Version 2.0 7-deaza-dGTP kit (US Biochemical, Cleveland, OH) according to the manufacturer's protocol. PCR products were purified and subcloned into plasmids (pBluescript SK-; Stratagene, San Diego, CA) with the TA subcloning system[13] and were prepared for sequence analysis. More than eight clones of each case were sequenced and compared with $V_H$ germline sequences published in the GenBank by means of the basic local alignment search tool program.[14] Observed and expected mutations were analyzed by using the following calculation: $R_{exp}$ or $S_{exp} = n \times (\text{CDR Rf or CDR Sf}) \times (CDR_{rel})$, where $R(S)_{exp}$ is the number of expected replacement (R) or silent (S) mutations, $n$ the number of observed mutations, R(S)f the proportion of total possible replacement (R) or silent (S) point mutations inherent in the CDR (complementarity-determining region) sequence, and $CDR_{rel}$ the proportion of CDR in the total $V_H$ sequence.[12, 15] This formula was also applied to the analysis of the FR (framework region). The probability P that the number of R mutations in the CDR occurred by chance was calculated with the formula of Chang and Casali: $P = \{n!/[k!(n-k)!]\}q^k(1-q)^{n-k}$, where $n$ is the number of observed mutations, $k$ the number of observed R mutations in the CDR or framework region(FR), and $q$ the probability that an R mutation will localize to CDR or FR ($q = (CDR_{rel}) \times (\text{CDR Rf})$ or $(FR_{rel}) \times (\text{FR Rf})$).[12, 15]

Table I. Patients Analyzed for Nucleotide Sequence

| Case number | Sex | Age | Histology[a] | Surface marker | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | CD5 | CD10 | CD19 | CD20 | DR |
| No. 1 | M | 51 | DLBL | + | − | + | + | + |
| No. 2 | F | 72 | DLBL | + | − | + | + | + |
| No. 3 | M | 57 | DLBL | + | − | + | + | + |
| No. 4 | F | 44 | DLBL | +/− | − | +/− | + | + |
| No. 5 | F | 60 | DLBL | − | + | + | + | + |
| No. 6 | F | 39 | DLBL | − | − | + | + | + |
| No. 7 | F | 55 | DLBL | − | − | − | + | ND |
| No. 8 | M | 52 | DLBL | − | − | + | + | + |
| No. 9 | M | 65 | MCL | + | − | + | + | + |
| No. 10 | M | 56 | MCL | + | − | + | + | + |
| No. 11 | M | 72 | B-CLL | + | − | + | + | + |

*a*) DLBL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; B-CLL, B-cell chronic lymphocytic lymphoma.

## RESULTS

**PCR amplification** The expected FR2-$J_H$ region was amplified by using consensus primers (FR2A, LJH and VLJH) in four of the 10 cases of $CD5^+$ DLBL, seven of the 10 cases of $CD5^-$ DLBL, two cases of MCL and one case of B-CLL. Four cases out of seven amplified $CD5^-$ DLBLs were randomly selected for sequence analysis.

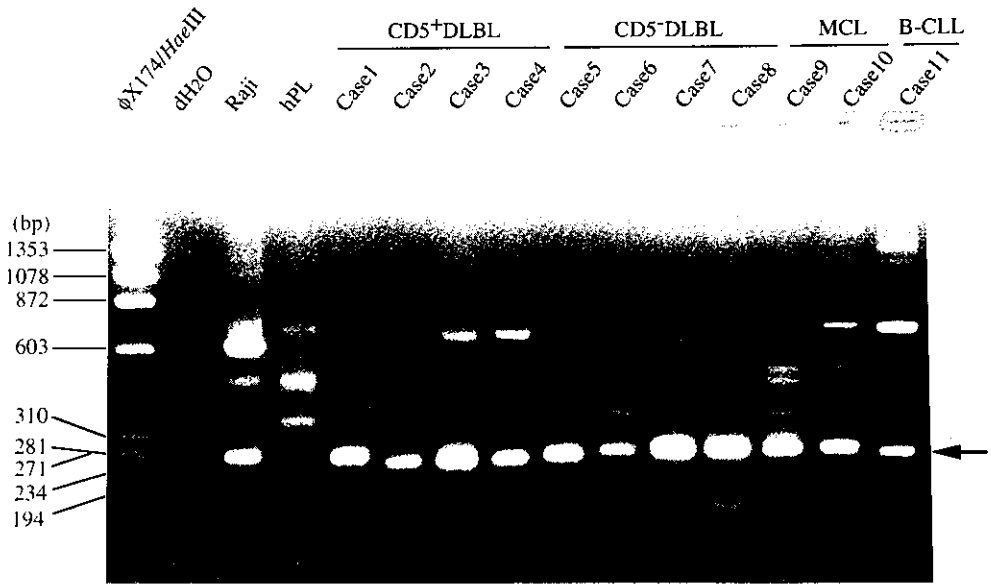


Fig. 1. Products of IgH PCR analyzed on 2.5% agarose gel stained with ethidium bromide. Raji, Raji DNA (positive control); hPL, human placental DNA (negative control); cases 1 through 4, CD5$^+$ DLBL cases; cases 5 through 8, CD5$^-$ DLBL cases; cases 9 and 10, MCL; case 11, B-CLL.

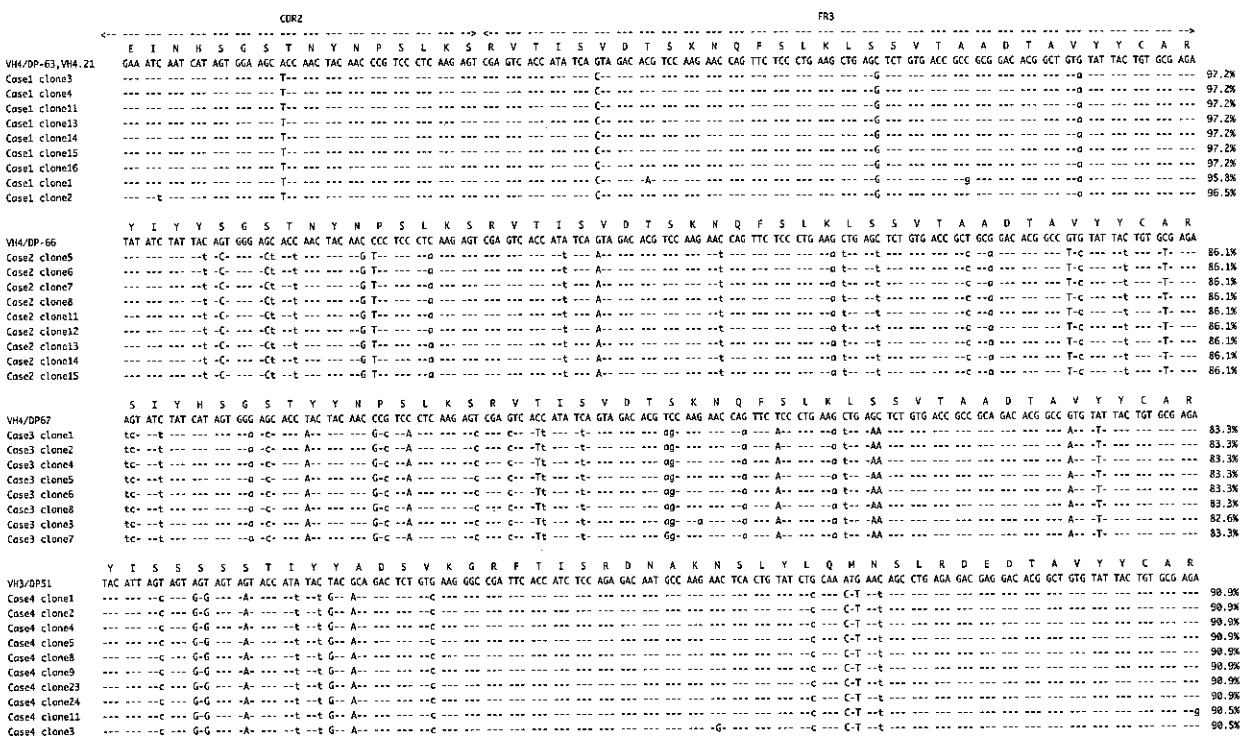


Fig. 2. Sequence analysis of the PCR products (CDR2-FR3 sequence) obtained from CD5$^+$ DLBL cases. The first line shows the amino acid sequences of germline genes. The second line shows the published $V_H$ germline genes most homologous to our sequencing data. The third and following lines show our sequencing data. Identity with the germline sequence is shown by dashes, replacement mutation by upper case letters, and silent mutation by lower case letters. The sequences of PCR primers (FR2A and VLJH) are not included.

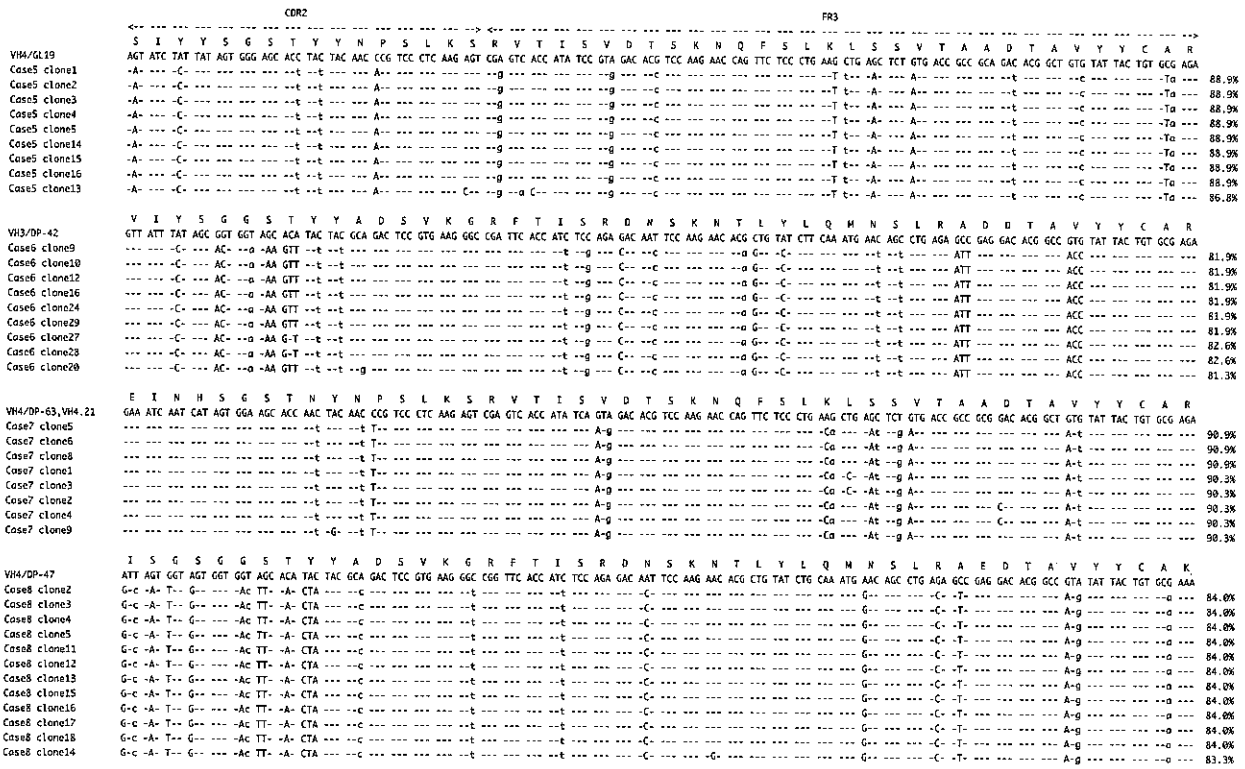

Fig. 3. Sequence analysis of the PCR products (CDR2-FR3 sequence) obtained from CD5$^-$ DLBL cases. See the legend to Fig. 2.

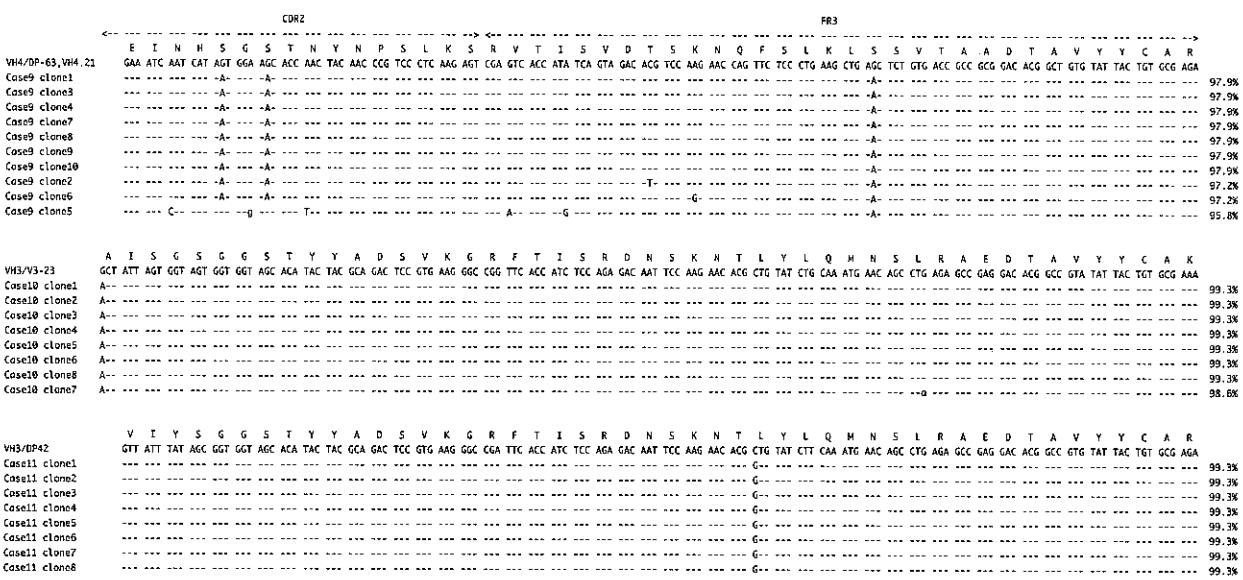

Fig. 4. Sequence analysis of the PCR products (CDR2-FR3 sequence) obtained from MCL and B-CLL cases. See the legend to Fig. 2.

Table II. Replacement (R) and Silent (S) Mutations in $V_H$ Segments of Rearranged Immunoglobulin Genes

| Case | Germline usage | Homology[a] (%) | CDR2[b] | | | | | FR3[c] | | | | | p-CDR2[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Expected | | Observed | | R/S ratio | Expected | | Observed | | R/S ratio | |
| | | | R | S | R | S | | R | S | R | S | | |
| No. 1 | VH4/DP63 | 97.2 | 1.1 | 0.3 | 1 | 0 | 1/0 | 1.9 | 0.7 | 2 | 1 | 2/1 | 0.4204 |
| No. 2 | VH4/DP66 | 86.1 | 5.3 | 1.3 | 4 | 4 | 4/4 | 9.7 | 3.6 | 3 | 9 | 3/9 | 0.1714 |
| No. 3 | VH4/DP67 | 83.3 | 6.5 | 1.5 | 3 | 7 | 3/7 | 11.7 | 4.3 | 6 | 8 | 6/8 | 0.0517 |
| No. 4 | VH3/DP51 | 90.9 | 3.6 | 0.9 | 5 | 4 | 5/4 | 6.5 | 2.0 | 2 | 2 | 2/2 | 0.1578 |
| No. 5 | VH4/GL19 | 88.9 | 4.3 | 1.1 | 3 | 2 | 3/2 | 7.7 | 2.9 | 4 | 7 | 4/7 | 0.1884 |
| No. 6 | VH3/DP42 | 81.9 | 6.8 | 2.2 | 8 | 3 | 8/3 | 13.8 | 4.2 | 9 | 7 | 9/7 | 0.1461 |
| No. 7 | VH4/DP63 | 90.9 | 3.5 | 0.8 | 1 | 2 | 1/2 | 6.3 | 2.3 | 5 | 5 | 5/5 | 0.0818 |
| No. 8 | VH4/DP47 | 84.0 | 5.8 | 1.8 | 11 | 4 | 11/4 | 11.8 | 3.6 | 5 | 3 | 5/3 | 0.0115 |
| No. 9 | VH4/DP63 | 97.9 | 0.8 | 0.2 | 2 | 0 | 2/0 | 1.5 | 0.5 | 1 | 0 | 1/0 | 0.1583 |
| No. 10 | VH3/V3-23 | 99.3 | 0.3 | 0.1 | 1 | 0 | 1/0 | 0.5 | 0.2 | 0 | 0 | 0/0 | 0.2625 |
| No. 11 | VH3/DP42 | 99.3 | 0.3 | 0.1 | 0 | 0 | 0/0 | 0.5 | 0.2 | 1 | 0 | 1/0 | 0.7470 |

*a*) Percent homology, seen in the dominant subclone of each case.
*b*) CDR, complementarity-determining region.
*c*) FR, framework region.
*d*) p, probability of obtaining the number of R mutations found in the CDR2 by chance.

The result of electrophoresis on 2.5% agarose gel of cases examined for sequencing is shown in Fig. 1.

**DNA sequence analysis** The DNA sequences obtained are shown in Figs. 2 through 4. Eight to 12 independent clones were sequenced from each case and more than six clones showed sequences suggesting the same clonal origin.

Fig. 2 shows that for $CD5^+$ DLBLs, three cases (nos. 1, 2 and 3) displayed the closest homology to the germline gene sequence of the $V_H4$ family, while case no. 4 displayed the closest homology to that of the $V_H3$ family. Sequences of these cases were frequently mutated and the percentage homology to their closest germline genes varied from 83.3% to 97.2%. All but one case (no. 2) included a few clones with additional mutations.

Fig. 3 summarizes the results for $CD5^-$ DLBLs. Cases no. 5, 7 and 8 showed the closest homology to the germline gene sequence of the $V_H4$ family and case no. 6 to that of the $V_H3$ family. The percentage homology of $CD5^-$ DLBL cases ranged from 81.9% to 90.9%. In case no. 7, three distinct subclones were observed. Subclones 5, 6 and 8 had ten mutations in the FR3 region, representing inherent sequences of this case, and the other two subclones had additional mutations. Similarly, in case no. 6, two subclones were observed.

MCL and B-CLL cases were studied as controls and the results are summarized in Fig. 4. Case no. 9 (MCL) used the germline genes of $V_H4$ and case nos. 10 (MCL) and 11 (B-CLL) displayed extremely close homology to the germline gene of $V_H3$. In addition, case no. 9 showed three nucleotide substitutions from the CDR2 through to the FR3 region. These substitutions all involve the second letter of the codons which code serine, with a G-to-A mutation resulting in replacement of serine with asparagine. Case nos. 10 (MCL) and 11 (B-CLL) expressed germline $V_H$ genes with one nucleotide substitution. The replacement and silent mutations in the $V_H$ segments are summarized in Table II. Regarding DLBL cases, R mutations observed in CDR are fewer than expected, except for case nos. 4, 6 and 8, and the R/S ratio is lower than 3.0, showing that DLBLs with or without CD5 were not implicated in antigen selection.

## DISCUSSION

Classification of malignant lymphomas employs several strategies. One elegant approach is to categorize the tumor cells according to their benign counterparts. This strategy is used by the Kiel classification.[16] Recently, a new scheme called the REAL classification has been proposed, which adopts the principle of classifying lymphomas according to their normal counterparts and, in addition, aims at obtaining new insights into tumor development by applying molecular biological techniques.[9] The histological category of DLBLs represents a heterogeneous group of non-Hodgkin's lymphomas of different origins, although it is difficult to delineate the morphologic subgroups by means of phenotypic and genotypic analyses. On the other hand, because of the poor reproducibility of histological examination and the absence of any significant clinical differences, the REAL classification does not recognize any subclassifications in this

category.[9] Therefore, it is important to examine if this category can be divided into subgroups by adopting newer molecular biological techniques.

Recently, it has been noted by Knowles' group and ours that *de novo* $CD5^+$ DLBLs appear to represent a neoplastic analogue of normal $CD5^+$ B cells and to constitute a distinct clinicopathologic and genotypic subgroup.[4,5] The present study has focused on the $V_H$ sequential aspect of these DLBLs to elucidate the biologic relationship with the other indolent $CD5^+$ B-cell lymphomas. Somatic mutation studies of the $V_H$ region by several groups have successfully defined the developmental stages of distinct types of leukemias and lymphomas. It was found that B-CLLs and MCLs lacked somatic hypermutation, suggesting that they are of the pre-GC stage.[6,7] DLBLs showed hypermutations, suggesting that they are of the post-GC stage, and follicular lymphomas featured hypermutations with on-going mutations, indicating that these are of the GC stage.[6,8] Although there have been reports of somatic mutation studies of DLBL, demonstrating that they are of the post-GC stage, there was no mention of the relation with CD5 expression.[6,17] One of these studies demonstrated that one out of 15 DLBLs showed 100% homology with a germline $V_H$ gene sequence, indicating this case was of pre-GC origin.[17] This case might have originated from the same developmental stage as that of MCLs and B-CLLs, although once again the relation with CD5 expression was not mentioned. Our results showing hypermutations ranging from 81.9 to 97.2% are in agreement with the results that DLBLs are derived from the post-GC stage irrespective of the presence of CD5 antigen. One case with 97.2% homology (case no. 1) was marginal in terms of percentage mutation. This $V_H$ sequence might represent an unpublished polymorphic germline sequence. This finding, considered in conjunction with the report of one case showing 100% identity with a $V_H$ germline, suggests the possibility that these less mutated cases might have developed from pre-GC stage cells identical to MCLs and B-CLLs. In this regard, it is interesting to note that our case was $CD5^+$. However, the rest of the $CD5^+$ DLBLs showed hypermutations, indicating that they were distinct from pre-GC lymphomas.

It is speculated that $CD5^+$ B cells belong to a different lineage (B1 cells) from normal B cells (B2 cells).[1,2] It is therefore possible that $CD5^+$ DLBLs do not proceed through the normal mutation stages of the germinal center, but that they might undergo somatic mutations somewhere other than germinal centers.[18] When the R/S ratio was calculated, none of the DLBL cases, irrespective of CD5 expression, gave a value of more than 3.0, the ratio indicating antigen selection (Table II). Furthermore, no significant differences were recognized between the two groups. Therefore, none of the indicators allowed us to distinguish the two groups from each other. The present study has therefore demonstrated that *de novo* $CD5^+$ DLBLs are immunogenotypically similar to $CD5^-$ DLBLs, but distinct from the other indolent $CD5^+$ B-cell lymphomas of CLL/SLL and MCL. We also confirmed the heterogeneity of $CD5^+$ B-cell neoplasms mentioned in previous reports. Although the clinicopathologic significance of this variant needs to be verified with a more extensive series, it may merit serious consideration at this early stage of investigation because of its aggressive clinical course and the unusual expression of CD5.

## ACKNOWLEDGMENTS

We would like to thank Drs. Y. Hosokawa, T. Joh and T. Akagi for valuable discussions, and to express our appreciation for the technical assistance of Ms. H. Suzuki, M. Sugiyama and Y. Maeda. This work was supported in part by a Grant-in-Aid for the Second-term Comprehensive Ten-Year Strategy for Cancer Control from the Ministry of Health and Welfare, a Grant-in-Aid for Scientific Research on Primary Areas (Cancer Research), a Grant-in-Aid for the Encouragement of Young Scientists from the Ministry of Education, Science and Culture, Japan, a Grant-in-Aid from the Japanese Foundation for Multidisciplinary Treatment of Cancer, and a grant from the Bristol-Myers Squibb Unrestricted Biomedical Research Grants Program.

*(Received July 31, 1997/Accepted September 22, 1997)*

## REFERENCES

1) Kipps, T. J. The CD5 B cell. *Adv. Immunol.*, **47**, 117–185 (1989).
2) Hayakawa, K., Hardy, R. R., Herzenberg, L. A. and Herzenberg, L. A. Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. *J. Exp. Med.*, **161**, 1554–1568 (1985).
3) Matolcsy, A., Chadburn, A. and Knowles, D. M. *De novo* CD5-positive and Richter's syndrome-associated diffuse large B cell lymphomas are genotypically distinct. *Am. J. Pathol.*, **147**, 207–216 (1995).
4) Knowles, D. M., II, Halper, J. P., Azzo, W. and Wang, C. Y. Reactivity of monoclonal antibodies Leu1 and OKT 1 with malignant human lymphoid cells. Correlation with conventional cell markers. *Cancer*, **52**, 1369–1377 (1983).
5) Yatabe, Y., Nakamura, S., Seto, M., Kuroda, H., Kagami, Y., Suzuki, R., Ogura, M., Kojima, M., Koshikawa, T., Ueda, R. and Suchi, T. Clinicopathological study of PRAD1/cyclin D1 overexpressing lymphoma with special reference to mantle cell lymphoma. A distinct molecular pathologic entity. *Am. J. Surg. Pathol.*, **20**, 1110–1122

(1996).
6) Hummel, M., Tamaru, J., Kalvelage, B. and Stein, H. Mantle cell (previously centrocytic) lymphomas express VH genes with no or very little somatic mutations like the physiologic cells of the follicle mantle. *Blood,* **84,** 403–407 (1994).
7) Küppers, R., Gause, A. and Rajewsky, K. B cells of chronic lymphatic leukemia express V genes in unmutated form. *Leuk. Res.,* **15,** 487–496 (1991).
8) Küppers, R., Rajewsky, K. and Hansmann, M.-L. Diffuse large cell lymphomas are derived from mature B cells carrying V region genes with a high load of somatic mutation and evidence of selection for antibody expression. *Eur. J. Immunol.,* **27,** 1398–1405 (1997).
9) Harris, N. L., Jaffe, E. S., Stein, H., Banks, P. M., Chan, J. K., Cleary, M. L., Delsol, G., De Wolf-Peeters, C., Falini, B., Gatter, K. C., Grogan, T. M., Isaacson, P. G., Knowles, T. M., Mason, D. Y., Muller-Hermelink, H. K., Pileri, S. A., Piris, M. A., Ralfkiaer, E. and Warnke, R. A. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood,* **84,** 1361–1392 (1994).
10) Nakamura, S., Seto, M., Banno, S., Suzuki, S., Koshikawa, T., Kitoh, K., Kagami, Y., Ogura, M., Yatabe, Y., Kojima, M., Motoori, T., Takahashi, T., Ueda, R. and Suchi, T. Immunohistochemical analysis on cyclin D1 protein in hematopoietic neoplasms with special reference to mantle cell lymphoma. *Jpn. J. Cancer Res.,* **85,** 1270–1279 (1994).
11) Seto, M., Yamamoto, K., Iida, S., Akao, Y., Utsumi, K. R., Kubonishi, I., Miyoshi, I.,Ohtsuki, T., Yawata, Y., Namba, M., Motokura, T., Arnold, A., Takahashi, T. and Ueda, R. Gene rearrangement and overexpression of PRAD1 in lymphoid malignancy with t(11;14)(q13;q32) translocation. *Oncogene,* **7,** 1401–1406 (1992).
12) Tamaru, J., Hummel, M., Marafioti, T., Kalvelage, B., Leoncini, L., Minacci, C., Tosi, P., Wright, D. and Stein, H. Burkitt's lymphomas express VH genes with a moderate number of antigen selected somatic mutations. *Am. J. Pathol.,* **147,** 1398–1407 (1995).
13) Sarkar, F. H., Ball, D. E., Li, Y. W. and Crissman, J. D. Molecular cloning and sequencing of an intron of Her-2/neu (ERBB2) gene. *DNA Cell Biol.,* **12,** 611–615 (1993).
14) Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. Basic local alignment search tool. *J. Mol. Biol.,* **215,** 403–410 (1990).
15) Chang, B. and Casali, P. The CDR1 sequences of a major proportion of human germline Ig $V_H$ genes are inherently susceptible to amino acid replacement. *Immunol. Today,* **15,** 367–373 (1994).
16) Lennert, K. and Feller, A. C. "Histopathology of Non-Hodgkin Lymphomas"(1992). Springer-Verlag, New York.
17) Hsu, F. J. and Levy, R. Preferential use of the VH4 Ig gene family by diffuse large-cell lymphoma. *Blood,* **86,** 3072–3082 (1995).
18) Murakami, M., Tsubata, T., Okamoto, M., Shimizu, A., Kumagai, S., Imura, H. and Honjo, T. Antigen-induced apoptotic death of Ly-1 B cells responsible for autoimmune disease in transgenic mice. *Nature,* **357,** 77–80 (1992).