HISTOGENESIS OF CD5-POSITIVE AND CD5-NEGATIVE B-CELL
NEOPLASMS ON THE ASPECT OF SOMATIC MUTATION
OF IMMUNOGLOBULIN HEAVY CHAIN
GENE VARIABLE REGION

NAOYA NAKAMURA and MASAFUMI ABE

*Department of Pathology I, Fukushima Medical University
School of Medicine, Fukushima*

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Abstract: The immunoglobulin heavy chain (IgH) gene of B-cells dramatically alters twice in their differentiation to memory or plasma cells; VDJ recombination at B-cell precursor and somatic hypermutation, class switch recombination and receptor revision at germinal center (GC) B-cells. Among them, somatic hypermutation of the IgH gene variable region (VH gene) is a powerful tool for detection of B-cell differentiation. B-cells and B-cell neoplasms have been divided into following; 1) pre-GC B-cells and neoplasms with a germline VH gene and 2) GC and post-GC B-cells and neoplasms with a somatically mutated VH gene. In this article, we review normal B-cell differentiation and histogenesis of various types of B-cell neoplasms on the aspect of somatic mutation of the rearranged VH gene. In particular, differences between CD5⁺ and CD5⁻ B-cell neoplasms, using our own data of over 100 cases with B-cell neoplasms, are discussed. Although CD5⁺ B-cells are included in pre-GC B-cells for the reason of germline VH gene in most of CD5⁺ B-cells, an about 5% of CD5⁺ B-cells show somatically mutated VH gene. The rearranged VH gene of CD5⁺ B-cell neoplasms shows heterogeneity, whereas CD5⁻ B-cell neoplasms possess somatically mutated VH gene with a mean of 8~12%. Both CD5⁺ B-cell chronic lymphocytic leukemia and CD5⁺ diffuse large B-cell lymphoma display that about half of cases show a germline or low frequency of somatic mutation and the others possess somatically mutated VH gene. CD5⁺ mantle cell lymphoma constitutes most cases with germline and a small number of cases with mutated VH gene. Therefore, CD5⁻ B-cells & CD5⁻ B-cell neoplasms are distinct from CD5⁺ B-cells and CD5⁺ B-cell neoplasms in somatic mutation of VH gene. It suggests that each of CD5⁻ and CD5⁺ B-cells independently has its own differentiation.

中村直哉, 阿部正文

Correspondence to: Naoya Nakamura, Department of Pathology, Fukushima Medical University School of Medicine, Fukushima City 960-1295, Japan.

E-mail: nao@fmu.ac.jp

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INTRODUCTION

Recent advance in the molecular biologic technique has provided new insights into biological characterization of B-cells and B-cell neoplasms¹⁻⁸. The availability of a complete map of immunoglobulin heavy chain (IgH) variable region gene (VH gene) repertoire and of the PCR technique for amplification of the VH gene allow us to analyze the degree of somatic mutation of the VH gene of B-cells. On this basis, the degree of somatic mutation of the VH gene depends on the level of B-cell differentiation during the immune response. It can be also possible to define the clonal origin of B-cell neoplasm by the analysis of rearranged VH gene.

Our purpose in this review is to clarify histogenesis of CD5-positive (+) and CD5-negative (-) B-cell neoplasms on the aspect of somatic mutation of the VH gene. We describe here 1) alteration of the IgH gene according to normal B-cell differentiation with peculiarity of somatic hypermutation of the VH gene, 2) the stereotype of normal B-cell differentiation and its problem, and finally 3) normal B-cell differentiation and histogenesis of CD5⁺ and CD5⁻ B-cell neoplasms.

OVERVIEW OF ALTERATION IN IMMUNOGLOBULIN HEAVY CHAIN GENE OF NORMAL B-CELLS

During normal B-cell differentiation, B-cells have two major phases with dramatic alteration for antigen molecule in the IgH gene^{1-7,9-11}. First, VDJ recombination of the Ig gene occurs in B-cell precursor in the bone marrow. Germlines of the IgH gene are comprised of 50 functional segments of Variable region, 27 segments of Diversity region and six segments of Joint region⁸. The Ig light (IgL) gene lacks D segments. D and J segments are first joined together and a V segment is subsequently rearranged to a DJ joining for generating antibody variants as shown in Fig. 1. The IgL genes are assembled from V and J elements (VJ recombination). In-frame rearrangements of VDJ in IgH and VJ in IgL are necessary for expression of Ig (B-cell receptor, BCR) and B-cells with the expression of antibody (BCR) can survive and differentiate. When the trial of VDJ recombination failed to make in-frame sequence, the second trial of the VDJ recombination is given on the other IgH allele.

B-cells with the expression of surface IgM and IgD (naïve B-cells) transfer to peripheral (secondary) lymphoid tissue of lymph nodes and spleen. Naïve B-cells around sheathed capillary in the spleen or paracortex in the lymph node are stimulated by binding cognate antigen with their antigen receptors and by interaction of T helper cells (antigen stimulation), following that B-cells form or migrate into

Fig.1. Alteration of the immunoglobulin heavy chain gene during B-cell differentiation, VDJ recombination, somatic hypermutation, class switching and receptor revision.

During normal B-cell differentiation, B-cells have two major phases with dramatic alteration for antigen molecule in the IgH gene. First, VDJ recombination of the Ig gene occurs in B-cell precursor in the bone marrow. Antigen-stimulated B-cells form or migrate into germinal center (GC) of secondary follicle. The B-cells proliferate several rounds and mature for increasing affinity of a BCR to the respective antigen by somatic hypermutation of the rearranged VH gene. Class switch recombination is undergone in the IgH gene of B-cells in the GC independent of somatic hypermutation. Changing the isotype of a BCR altered effector functions of the antibody. A BCR is replaced by C γ (IgG), C α (IgA) or C ϵ (IgE) instead of C μ (IgM) and C δ (IgD). Receptor revision (editing) is also a process by which an originally expressed antibody polypeptide chain, usually the IgL is replaced by another one.

germinal center (GC) of secondary follicle. In GC, antigen-stimulated B-cells proliferate several rounds and mature for increasing affinity of a BCR to the respective antigen (affinity maturation). Affinity maturation is achieved by nucleotide substitutions of the rearranged VH gene. They are randomly introduced into the VH gene at a very high rate (10^{-3} to 10^{-4} /bp/generation) and called as somatic hypermutation. B-cells with high affinity of a BCR for a distinct antigen can survive by contact to follicular dendritic cells (FDCs), escape outside GC and finally differentiate into memory B-cells or plasma cells. When B-cells fail to increase affinity or have a BCR with reactive for self-antigen, these cells proceed to apoptotic death. Macrophages immediately clean the dead cells off the GC. Class switch recombination is undergone in the IgH gene of B-cells in the GC independent of somatic hypermutation. Changing the isotype of a BCR altered effector functions of the antibody. Before entering into GC, B-cells express C μ (IgM) and C δ (IgD). A BCR is replaced by C γ (IgG), C α (IgA) or C ϵ (IgE). Receptor revision (editing) is also a process by which an originally expressed antibody polypeptide

chain, usually the IgL is replaced by another one.

These mechanism, VDJ recombination, somatic hypermutation, class switch and receptor revision are closely related to oncogenesis of B-cell lymphoma. These remodelings of the Ig gene are explained as leading double strand breaks to the Ig gene, so that chromosomal translocation involving Ig gene may happen in both two stages of B-cell progenitors and GC B-cells. For examples, translocation of the *bcl-2* gene and the IgH gene; t(14; 18)(q32; q21) is speculated to occur during VDJ recombination in B-cells progenitors in the bone marrow, because a breakpoint of the IgH gene is located in VDJ segments¹²⁾. Translocation of the *c-myc* gene and the IgH gene; t(8; 14)(q24; q32) may generate during the class switch recombination in GC B-cells because a breakpoint of the IgH gene is located in C segments¹³⁾.

PECULIARITY AND ANALYSIS-METHODS OF SOMATIC MUTATION OF THE IGH GENE

Somatic hypermutation of the VH gene is found in a region about 1-2 kb downstream of the transcriptional promoter, so that somatic hypermutation depends on Ig enhancers and on transcription of the Ig gene. This unique mechanism is not a misreading in DNA synthesis, but associated with double-stranded break as similar as VDJ recombination and class switch. The *bcl-6* gene subsequently reported to show somatic mutation on GC B-cells and some B-cell neoplasms¹³⁾. Diffuse large B-cell lymphoma (DLBCL) possessed somatic mutation not only in VH and *bcl-6* genes, but also the other several genes such as *c-myc* and *pim-1*¹⁴⁾. Somatic mutation frequencies in these genes other than the VH gene are, however, very low in comparison to the VH gene.

The VH gene is comprised of Frame Work (FW) 1, Complementary Determinating Region (CDR) 1, FW2, CDR2, FW3 and a part of CDR3. CDR3 constitutes V-N-D-N-J. Random nucleotides are inserted to two sites between V and D and between D and J by Terminal deoxy-Transferase (TdT) activity, so B-cells have a variable length of 300~400 bps VH gene dependent on their VDJ recombination¹⁵⁾. The VH gene can be amplified using DNA from cell suspension, frozen material, as well as paraffin-embedded material. The VH gene is normally amplified by two times PCR methods, such as semi-nested PCR using FR1C as sense primer and LJH and VLJH as anti-sense primers¹⁶⁾. Because a length of PCR-amplified VH gene of each B-cell depends on its VDJ rearrangement, a size of PCR product of each B-cell is different. An amplified single and discrete band indicates a clonal proliferation of VDJ rearranged B-cells, whereas smear bands suggest non-neoplastic B-cells. If primers failed to anneal a template DNA because of mutations in the primers area of the template DNA, a VH gene may not be amplified. A polyacrylamide gel electrophoresis with 4-6% concentration instead of agarose gel electrophoresis is needed for measuring precise size of the PCR products or comparing two samples.

Nucleotide sequences of the amplified IgH gene are analyzed by a direct sequence method of the PCR products. Alternatively, the PCR products are cloned

to an appropriate vector devised for the cloning of the PCR products and sequenced. The nucleotide sequences analyzed are compared to registered IgH germlines in Gene banks such as the Genbank in the National Cancer Institute (<http://www.ncbi.nlm.nih.gov/blast/> or <http://www.ncbi.nlm.nih.gov/igblast/>). The closest germline with highest homology to the nucleotide sequence of examined case is regarded as the germline usage for the case. Somatic mutations of two types exist. Replacement (R) mutation is single-base substitutions resulting in amino acid replacement and silent (S) mutation is single-base substitutions without amino acid replacement. Somatic mutation frequency represents as percentage of the number of R- and S- mutations divided the number of examined nucleotides. Insertions and deletions may be observed. The EB virus transformed peripheral blood lymphocytes cell lines show a VH gene utilization roughly proportional to the estimated family sized and functional VH genes dispersed into 7 families and germline complexity of these family is reported to be 25-30% of VH1 family, 5-10% of VH2 family, 50-55% of VH3 family, 5-18% of VH4 family, 1-2% of VH5 family and 1% of VH6 family¹⁷⁾.

B-CELL DIFFERENTIATION AND HISTOGENESIS OF B-CELL NEOPLASMS

1) *The stereotype and its problem*

Fig. 2 shows differentiation of normal B-cells with their immunophenotype and counterpart cells of various types of B-cell neoplasms. It is mainly explained by changing of immunophenotypic markers of CD5, CD10 and CD38. The aspect of somatic mutation of the VH gene has given the concept that normal B-cells are divided into antigen-stimulated B-cells and un-stimulated B-cells. Un-stimulated B-cells situate at pre GC. The rearranged VH gene of pre-GC B-cells, consisting of precursor B-cells and naïve B-cells, shows germline. Antigen-stimulated B-cells mean GC B-cells and post GC B-cells including memory B-cells and plasma cells, and both GC B-cells and post GC B-cells possess a somatically mutated VH gene. Somatic mutation analysis of the rearranged VH genes of neoplastic B-cells has been extensively published so far and that theory seems to be generally accepted for B-cell neoplasms. B-cell neoplasms derived from pre-GC B-cells have an unmutated VH gene, whereas those derived from GC B-cells and post GC B-cells have a hypermutated VH gene.

Several questions, however, remain. The most important question, which is our purpose of this article, is the existence of a unique system of CD5⁺ B-cells. Although CD5 is regarded as a differentiation marker in pre-GC B-cells and CD5⁺ B-cells were set in pre-GC naïve B-cells (Fig. 2), it may be incorrect. We have investigated a large scale of somatic mutation analysis in the rearranged VH gene of B-cell neoplasms and literature review for normal B-cells with a special attention to CD5⁺ and CD5⁻ B-cells and their neoplasms. Somatic mutation frequencies of various types of B-cell neoplasms are shown in Table 1 and the histogram of somatic

Fig. 2. Differentiation of B-cells and histogenesis of B-cell neoplasms. (stereotype)

During normal B-cell differentiation, antigen-stimulated naive B-cells form or migrate into germinal center (GC) of secondary follicle. In GC, antigen-stimulated B-cells proliferate and mature for increasing affinity of a BCR to the respective antigen (affinity maturation) achieved by somatic hypermutation of the rearranged VH gene. B-cells with high affinity of a BCR for a distinct antigen can survive by contact to follicular dendritic cells (FDCs), escape outside GC and finally differentiate into memory B-cells or plasma cells. When B-cells fail to increase affinity or have a BCR with reactive for self-antigen, these cells proceed to apoptotic death.

B-CLL/SLL, B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma; MCL, mantle cell lymphoma; PCM, plasma cell myeloma; MZBCL, marginal zone B-cell lymphoma; BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma.

Table 1. Somatic mutation frequencies of the VH gene of various types of B-cell neoplasms

	examined cases	distribution (%)	mean (%)
Lymphoblastic lymphoma (LBL)	6	0- 0.5	0.1
Chronic lymphocytic leukemia (B-CLL)	11	0-15.0	6.8
Mantle cell lymphoma (MCL)	11	0- 6.8	2.1
Follicular lymphoma (FL)	5	7.9-12.9	10.2
Diffuse large B-cell lymphoma (DLBCL)	39	0.9-25.9	10.6
Burkitt lymphoma (BL)	7	0.5- 9.8	4.5
MALT lymphoma	11	6.1-12.6	8.6
Plasma cell myeloma (PCM)	5	4.8-16.3	9.0

mutation frequencies of each B-cell neoplasm is shown in Fig. 3.

2) CD5⁺ B-cells and CD5⁺ B-cell neoplasms

CD5⁺ B-cells, which are called B-1 cells, are known to constitute of the major subpopulation of B-cells by their distinct differences to CD5⁻ B-cells in anatomic

Fig. 3. Histogram of somatic mutation frequency of CD5⁺ and CD5⁻ B-cell neoplasms.

CD5⁺ B-cell neoplasms (upper) versus CD5⁻ B-cell neoplasms (lower).

Each of CD5⁺ B-cell neoplasms indicates heterogeneous pattern, whereas all of CD5⁻ B-cell neoplasms are somatically hypermutated. The histogram of CD5⁻ DLBCL excluded DLBCLs with more than 16%-mutation frequency.

localization, immunophenotype, gene usage and function¹⁸⁻²²). The CD5 antigen is expressed on varying number of B-cells throughout life; 40-60% in fetus, 20% in adult peripheral blood and spleen, and 30% in adult lymph nodes and tonsils^{19,20}. In secondary lymphoid tissue such as the lymph node, CD5⁺ B-cells locate in mantle zone of secondary follicle, but not in GC. CD5⁺ B-cells constitute a distinct developmental lineage and may produce a disproportionate level of low affinity and poly-specific autoantibodies^{18,21}. Differences in selection and somatic mutations between CD5⁺ B-cells and CD5⁻ B-cells have been also reported²³. Although most CD5⁺ B-cells carry non-mutated VH and VL genes, indicating that CD5⁺ B-cells are naïve B-cells, about 5% of PB CD5⁺ B-cells possess somatically mutated VH gene and co-express CD27, memory B-cell marker²²). Moreover, somatic mutations in autoantibody-associated VH genes of circulating IgM⁺ IgD⁺ B cells have been described²⁴). Thus, CD5⁺ B-cells are comprised of naïve B-cells and memory B-cells.

CD5⁺ B-cell neoplasms are heterogeneous in morphology, immunophenotype and genotype. Most cases of B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma (B-CLL/SLL) and mantle cell lymphoma (MCL), some cases of hairy cell leukemia (HCL) and 5-10% of diffuse large B-cell lymphoma (DLBCL) are known to express the CD5 antigen²⁵⁻²⁷).

The rearranged VH genes of B-CLL/SLL are not uniform. Although B-CLL was initially considered to lack a significant hypermutation of the rearranged VH gene, CD5⁺ B-CLL with a somatically mutated V gene has subsequently been

described²⁸⁾. The frequency of somatic mutation of CD5⁺ B-CLL/SLL in our series varied from 0% to 15.0%, and B-CLL is apparently divided into two groups; cases with germline VH gene showing less than 2%-mutation frequency and cases with somatically mutated VH gene that distribute from 2% to 15%²⁹⁾. The B-CLL/SLL cells may arise from CD5⁺ B-cells that are exposed to a self-antigen, and produce auto-reactive antibodies³⁰⁾, and the proportion of B-CLL with a somatically mutated VH gene is estimated at 40% of B-CLL²²⁾.

MCL has the morphologic and immunologic profiles of tumor cells derived from B-cells of the secondary follicle mantle zone³¹⁾, and it is described as a B-cell neoplasm with the germline of a rearranged VH gene. It is widely accepted that CD5⁺ B-cells (naive B-cells) situated in the mantle zone with a germline VH gene are the normal counterpart of MCL. However, more than two-thirds of cases with MCL have a germline sequence within 2% -mutation-frequency, whereas the others showed 2.0%~6.8%³²⁾. We recently found the latter cases were memory B-cell MCLs. The case, interestingly, shared antigens of CD20, CD5, cyclin D1, a definite marker of MCL, and CD27, memory B-cell marker³³⁾. Somatic mutation frequency of the VH gene of this case was 6.8%. The existence of such memory B-cell MCL supports that B-cell neoplasms from CD5⁺ B-cells are comprised of both B-cells with a germline VH gene and with a mutated VH gene.

Several reports on the analysis of somatic mutations in CD5⁺ DLBCLs have showed that most cases are somatically mutated^{32,34-36)}. CD5⁺ DLBCL as well as CD5⁺ B-CLL showed various degrees of somatic mutation with a range of 0.7~12.9%³¹⁾. An average of frequency of somatic mutation of CD5⁺ DLBCL was also similar to that of CD5⁺ B-CLL³¹⁾. A Richter transformation must be developed from the clone of a preexisting B-CLL, so it is likely that the tumor clone of a Richter transformation showed little mutation of the rearranged VH gene. De novo CD5⁺ DLBCLs with little mutated VH gene were demonstrated (Fig. 4). CD5⁺ DLBCLs with somatically mutated VH genes were also documented in Western countries³⁶⁾. CD5⁺ DLBCL, as well as CD5⁺ B-CLL, may be divided into two groups of germline or low frequency group (0-5%) and somatically hypermutated group (5-17%) see Fig. 4. Therefore, the common characteristic of the rearranged VH gene of each CD5⁺ B-cell neoplasm is its heterogeneity.

To compare with germline complexity of VH gene family¹⁷⁾, the VH gene family usage of both CD5⁺ B-CLL and CD5⁺ DLBCL was biased. More cases of CD5⁺ B-CLL in the Western have VH5 and VH6 families and it is suggested that this biased VH gene usage is related to antigen selection by autoantigens. Ikematsu has reported that CD5⁺ B-CLL in the Japanese represents high proportion of VH4 usage when compared with the respective gene complexity for each VH family³⁷⁾. CD5⁺ B-CLLs with VH4 family constituted 40% of our cases, so high proportion of VH4 usage of the Japanese cases is considered to be characteristics. A half of CD5⁺ DLBCLs had VH4 family. Thus, this could be one of common characteristics between CD5⁺ DLBCL and CD5⁺ B-CLL in the Japanese population.

Fig. 4. Histogram of somatic mutation frequency of CD5⁺ and CD5⁻ diffuse large B-cell lymphomas (DLBCL).

CD5⁺ DLBCL shows heterogeneity, whereas CD5⁻ DLBCL has a peak of somatically mutated VH gene.

3) CD5⁻ B-cells and CD5⁻ B-cell neoplasms

Eighty-five percents of PB B-cells are of CD5⁻negative²²⁾. About half of CD5⁻ B-cells have unmutated VH gene, whereas the others have a somatically mutated VH gene with a mean of 5-6% mutation frequency. The IgM⁺ IgD⁺ subpopulation expressing CD27 carry somatically mutated V gene³⁸⁾. In secondary lymphoid tissue, GC B-cells express CD5⁻ and CD10⁺ and have somatically mutated VH gene with a similar average. The VH genes of marginal zone B-cells and plasma cells are somatically mutated²²⁾.

CD5⁻ B-cells neoplasms must be divided into B-LBL and other than B-LBLs. B-LBL is distinct from other CD5⁻ B-cell neoplasms because B-LBLs are derived from precursor B-cells. B-LBL is the same disease entity as precursor B-cell acute lymphoblastic leukemia, both of which express CD79a, CD19, CD20 and CD10 in various number, but not surface Ig nor CD5³⁹⁾. These disorders involve mainly bone marrow and PB. The IgH gene in most of B-LBL is rearranged and shows germline sequence⁴⁰⁾. CD5⁻ B-cells neoplasms other than B-LBL are mature B-cell neoplasms derived from GC and post GC B-cells, so most of them possess somatically mutated VH gene^{27,41)}. FL is believed to be GC B-cell neoplasms⁴¹⁾. About 80% of FLs have a *bcl-2/Ig* translocation, t(14; 18)(q32; q21), which occurs at VDJ rearrangements³⁹⁾. They exclusively express CD79a and CD10 and constitute follicular pattern with FDC network. Because GC B-cells show intraclonal diversity by ongoing somatic hypermutation, the rearranged VH gene of FLs possessed somatic hypermutation and intraclonal microheterogeneity (ongoing mutation)⁴²⁾. DLBCL constitutes the largest category accounting for 30-40% of all lymphoma

cases among Japan, Korea and Western countries³⁹). DLBCLs show heterogeneity in their biologic properties. DLBCLs express GC B-cells and/or post-GC B-cell markers. Gene abnormalities of DLBCLs, except CD5⁺ DLBCL, are believed to occur in GC⁴³) and most reports of somatic mutation of the rearranged VH gene of CD5⁻ DLBCLs confirm its oncogenesis. A small number of DLBCLs have extremely high percentages of somatic mutation frequencies more than 15%²⁷). This phenomenon is unknown, but additional mutation in the VH gene may occur during lymphoma cell expansion. MALT lymphoma (=extranodal marginal zone B-cell lymphoma, MZBCL) arises in mainly extranodal site and rarely in lymph node and spleen, which are called as nodal and splenic MZBCL, respectively³⁹). MALT lymphomas also possess a somatically mutated VH gene⁴⁴). The rearranged VH gene of splenic MZBCL may be or may be not mutated⁴⁵), but the origin of unmutated splenic MZBCL is unknown. PCM is a neoplasm derived from plasma cells situated at the end stage of B-cell differentiation and a rearranged VH gene of PCM is also somatically mutated⁴⁶). Each of a mean of somatic mutation frequencies of FL, CD5⁻ DLBCL, MALT lymphoma and PCM are 8~11%. Although BL is a B-cell neoplasm that derived from GC B-cells, somatic mutation frequencies of BL are somewhat different from other CD5⁻ B-cell neoplasms⁴⁷). They are lower and distributed between 0.5~10.0%. BLs are generated by translocation of the *c-myc* gene and the IgH gene; t(8; 14) (q24; q32) during the class switch recombination in GC B-cells⁴⁸). BLs have CD10 and a marker of early GC phase and lower percentage of somatic mutation frequency of rearranged VH gene, so that GC B-cells at early phase of differentiation is counterpart cells of BL.

4) *Appendix, Hodgkin lymphoma*

Hodgkin/Reed-Sternberg (HRS) cells were suggested to be B-cells, because a small percentage of HRS cells express B-cell antigen such as CD20 in immunohistochemistry, and Southern blotting with the IgH gene probe infrequently revealed a rearrangement band in a sample of HL. Recent molecular techniques of single cell PCR have confirmed B-cell origin of HRS cells⁴⁹). PCR amplification of the IgH gene from micro-dissected HRS cells from the HL frozen sections elegantly demonstrated monoclonal proliferation of B-cells with somatically mutated VH gene. Out-of-frame sequence of the rearranged VH gene is found in HRS cells. Those data indicated GC B-cells are the counterpart cells of HRS cells.

5) *Conclusion*

The common characteristic of CD5⁺ B-cell neoplasms is heterogeneity of somatic mutation pattern in the VH gene, whereas that of CD5⁻ B-cell neoplasm is a somatically hypermutated VH gene. CD5⁺ B-cells and CD5⁻ B-cells can independently differentiate and CD5⁺ B-cell neoplasms and CD5⁻ B-cell neoplasms are distinct in somatic mutation pattern of the IgH gene (Fig. 5).

Fig. 5. Differentiation of B-cells and histogenesis of B-cell neoplasms.

This figure shows that differentiation of CD5⁺ B-cells and CD5⁻ B-cells are independent.

(Modified, Klein U et al.¹²⁹)

REFERENCES

1. Tonegawa S. Somatic generation of antibody diversity. *Nature*, **302**: 575-581, 1983.
2. Rajewsky K. Clonal selection and learning in the antibody system. *Nature*, **381**: 751-758, 1996.
3. Lam KP, Kuhn R, Rajewsky K. In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. *Cell*, **90**: 1073-1083, 1997.
4. Nussenzweig MC. Immune receptor editing: revise and select. *Cell*, **95**: 875-878, 1998.
5. Liu YJ, Joshua DE, Williams GT, Smith CA, Gordon J, MacLennan IC. Mechanism of antigen-driven selection in germinal centers. *Nature*, **342**: 929-931, 1989.
6. Jacob J, Kelsoe G, Rajewsky K, Weiss U. Intracloonal generation of antibody mutants in germinal centers. *Nature*, **354**: 389-392, 1991.
7. Berek C, Ziegner M. The maturation of immune response. *Immunol Today*, **14**: 400-404, 1993.
8. Cook GP, Tomlinson IM. The human immunoglobulin VH repertoire. *Immunol Today*, **16**: 237-242, 1995.
9. Goossens T, Klein U, Kuppers R. Frequent occurrence of deletions and duplications during somatic hypermutation: implications for oncogene translocations and heavy chain disease. *Proc Natl Acad Sci USA*, **95**: 2463-2468, 1998.
10. Hikida M, Mori M, Takai T, Tomochika K, Hamatani K, Ohmori H. Reexpression of RAG-1 and RAG-2 genes in activated mature mouse B cells. *Science*, **274**: 2092-2094, 1996.
11. Liu YJ, Arpin C, de Bouteiller O, Guret C, Banchereau J, Martinez-Valdez H, Lebecque S. Sequential triggering of apoptosis, somatic mutation and isotype switch during germinal center development. *Semin Immunol*, **8**: 169-177, 1996.
12. Küppers R, Dalla-Favera R. Mechanisms of chromosomal translocations in B cell lymphomas. *Oncogene*, **20**: 5580-5594, 2001.
13. Shen HM, Peters A, Baron B, Zhu X, Storb U. Mutation of BCL-6 gene in normal B cells

- by the process of somatic hypermutation of Ig genes. *Science*, **280**: 1750-1752, 1998.
14. Pasqualucci L, Neumeister P, Goossens T, Nanjangud G, Chaganti RS, Kuppers R, Dalla-Favera R. Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. *Nature*, **412**: 341-346, 2001.
 15. Diaz Cano S. PCR-based alternative for diagnosis of immunoglobulin heavy chain gene rearrangement: principles, practice, and polemics. *Diagn Mol Pathol*, **5**: 3-9, 1996.
 16. Kuze T, Nakamura N, Hashimoto Y, Abe M. Most of CD30+ anaplastic large cell lymphoma of B cell type show a somatic mutation in the IgH V region genes. *Leukemia*, **12**: 753-757, 1998.
 17. Stewart K, Schwartz RS. Immunoglobulin V regions and the B cell. *Blood*, **83**: 1717-1730, 1994.
 18. Hayakawa K, Hardy RR, Herzenberg LA. Progenitors for Ly-1 B-cells are distinct from progenitors for other B cells. *J Exp Med*, **161**: 1554-1568, 1985.
 19. Hardy RR, Hayakawa K. Development and physiology of LY-1 B and its human homolog, Leu-1B. *Immunol Rev*, **93**: 53-79, 1986.
 20. Kipps TJ. The CD5 B cells. *Adv Immunol*, **47**: 117-185, 1989.
 21. Stall AM, Wells SM, Lam KP. B-1 cells: unique origin and functions. *Semin Immunol*, **8**: 45-59, 1996.
 22. Klein U, Goossens T, Fischer M, Kanzler H, Braeuninger A, Rajewsky K, Küppers R. Somatic hypermutation in normal and transformed human B cells. *Immunol Rev*, **162**: 261-280, 1998.
 23. Brezinschek HP, Foster SJ, Brezinschek RI, Dorner T, Domiati Saad R, Lipsky PE. Analysis of the human VH gene repertoire. Differential effects of selection and somatic hypermutation on human peripheral CD5(+)/IgM+ and CD5(-)/IgM+ B cells. *J Clin Invest*, **99**: 2488-2501, 1997.
 24. Munakata Y, Saito S, Hoshino A, Muryoi T, Hirabayashi Y, Shibata S, Miura T, Ishii T, Funato T, Sasaki T. Somatic mutation in autoantibody-associated VH genes of circulating IgM+IgD+ cells. *Eur J Immunol*, **28**: 1435-1444, 1998.
 25. Burns BF, Warnke RA, Doggett RS, RousRV. Expression of a T-cell antigen (Leu-1) by B-cell lymphoma. *Am J Pathol*, **113**: 165-171, 1983.
 26. Knowles DM, Halper JP, Azzo W, Wang CY. Reactivity of monoclonal antibodies Leu 1 and OKT1 with malignant human lymphoid cells: correlation with conventional cell markers. *Cancer*, **52**: 1369-1377, 1983.
 27. Zukerberg LR, Medeiros LJL, Ferry JA, Harris NL. Diffuse low-grade B-cell lymphomas: four clinically distinct subtypes defined by a combination of morphologic and immunophenotypic features. *Am J Clin Pathol*, **100**: 373-385, 1993.
 28. Oscier DG, Thompsett A, Zhu D, Stevenson FK. Differential rates of somatic hypermutation in VH genes among subsets of chronic lymphocytic leukemia defined by chromosomal abnormalities. *Blood*, **89**: 4153-4160, 1997.
 29. Nakamura N, Kuze T, Hashimoto Y, Tasaki K, Hojo H, Sasaki Y, Sato M, Abe M. Analysis of the immunoglobulin heavy chain gene variable region of 101 cases with peripheral B-cell neoplasms in the Japanese population. *Pathol International*, **49**: 595-600, 1997.
 30. Caligaris-cappio F. B-chronic lymphocytic leukemia: A malignancy of anti-self B-cells. *Blood*, **87**: 2615-2620, 1996.
 31. Weisenburger DD, Armitage JO. Mantle cell lymphoma -an entity comes of age. *Blood*, **87**: 4483-4494, 1996.
 32. Nakamura N, Hashimoto Y, Kuze T, Tasaki K, Sasaki Y, Sato M, Abe M. Analysis of the immunoglobulin heavy chain gene variable region of CD5+ positive diffuse large B-cell lymphoma. *Lab Invest*, **79**: 925-933, 1999.
 33. unpublished data
 34. Kume M, Suzuki R, Yatabe Y, Kagami Y, Miura I, Miura AB, Morishima Y, Nakamura S, Seto M. Somatic hypermutation in the VH segment of immunoglobulin genes of CD5-

- positive diffuse large B-cell lymphoma. *Jpn J Cancer Res*, **88**: 1087-1093, 1997.
35. Taniguchi M, Oka K, Hiasa A, Yamaguchi M, Ohno T, Kita K, Shiku H. De novo CD5⁺ diffuse large B-cell lymphomas express VH genes with somatic mutation. *Blood*, **91**: 1145-1151, 1998.
 36. Katzenberger T, Lohr A, Schwarz S, Dreyling M, Schoof J, Nickenig C, Stilgenbauer S, Kalla J, Ott MM, Muller-Hermelink HK, Ott G. Genetic analysis of de novo CD5⁺ diffuse large B-cell lymphomas suggests an origin from a somatically mutated CD5⁺ progenitor B cell. *Blood*, **101**: 699-702, 2003.
 37. Ikematsu W, Ikematsu H, Okamura S, Otsuka T, Harada M, Niho Y. Surface phenotype and Ig heavy-chain gene usage in chronic B-cell leukemias: expression of myelomonocytic surface markers in CD5⁻ chronic leukemia. *Blood*, **83**: 2602-2610, 1994.
 38. Klein U, Rajewsky K, Küppers R. Human immunoglobulin (Ig)M+IgD⁺ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. *J Exp Med*, **188**: 1679-1689, 1998.
 39. Jaffe ES, Harris NL, Stein H, Varidiman JW (eds.) World Health Organization Classification of Tumours. Tumours of Haematopoietic and Lymphoid tissues. IARC press Lyon 2002.
 40. Hojo H, Sasaki Y, Nakamura N, Abe M. Absence of somatic hypermutation of immunoglobulin heavy chain variable region genes in precursor B-lymphoblastic lymphoma: a study of four cases in childhood and adolescence. *Am J Clin Pathol*, **116**: 673-682, 2001.
 41. Müller-Hermelink HK, Greiner A. Molecular analysis of human immunoglobulin heavy chain variable gene (IgVH) in normal and malignant B cells. *Am J Pathol*, **153**: 1341-1345, 1998.
 42. Bahler DW, Levy R. Clonal evolution of follicular lymphoma: Evidence for antigen selection. *Proc Natl Acad Sci USA*, **89**: 6770-6774, 1992.
 43. Küppers R, Klein U, Hansmann ML, Rajewsky K. Cellular origin of human B-cell lymphomas. *N Engl J Med*, **341**: 1520-1529, 1999.
 44. Qin Y, Greiner A, Trunk MJ, Schmausser B, Ott MM, Muller-Hermelink HK. Somatic hypermutation in low-grade mucosa-associated lymphoid tissue-type B-cell lymphoma. *Blood*, **86**: 3528-3534, 1995.
 45. Algara P, Mateo MS, Sanchez-Beato M, Mollejo M, Navas IC, Romero L, Sole F, Salido M, Florensa L, Martinez P, Campo E, Piris MA. Analysis of the IgV(H) somatic mutations in splenic marginal zone lymphoma defines a group of unmutated cases with frequent 7q deletion and adverse clinical course. *Blood*, **99**: 1299-1304, 2002.
 46. Bakkus MH, Heirman C, Van Reith I, Van Camp B, Thielemans K. Evidence that multiple myeloma Ig heavy chain VDJ genes contain somatic mutations but show no intraclonal variation. *Blood*, **80**: 2326-2335, 1992.
 47. Nakamura N, Nakamine H, Tamaru J, Nakamura S, Yoshino T, Ohshima K, Abe M. The Distinction between Burkitt Lymphoma and Diffuse Large B-Cell Lymphoma with *c-myc* Rearrangement. *Mod Pathol*, **15**: 771-776, 2002.
 48. Hecht JL, Aster JC. Molecular Biology of Burkitt's Lymphoma. *J Clin Oncol*, **18**: 3707-3721, 2000.
 49. Küppers R. Molecular biology of Hodgkin's lymphoma. *Adv Cancer Res*, **84**: 277-312, 2002.