

Somatic mutation in rearranged IgHV gene in various B-cell malignancies

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Recent progress in the molecular biological characterization of the B cell response and the introduction of the polymerase chain reaction (PCR) in connection with direct sequencing procedures of the PCR amplicates, the normal counterpart of various B cell malignancies can now be investigated. It has been shown that pre-germinal center B cells, ie, immature sIg M+ B cells and naive mature antigen reactive sIg M+ sIg D+ B cells express Ig V region genes with a germ line sequence in contrast to germinal center (GC) B cells and post-GC B cells that contain mutated V region genes. GC B cells and post-GC B cells can be distinguished from each other, because in GC B cells, the mutation process is usually ongoing, whereas in post-GC B cells this process is switched off.

To help clarify whether various B cell malignancy are related to nonmutated pre-GC B cells or mutated GC B cells or post-GC B cells, we amplified the IgH VDJ rearrangement employing extracted DNA from various cases of B cell malignancies, and analyzed the sequences for the presence of somatic mutations. To detect the ongoing somatic mutation, the amplicates were subcloned and the each sequence was compared.

Materials and Methods

Tissue samples

Formalin-fixed/paraffin-embedded bioptic specimens of the following tissues were investigated: six cases of B-chronic lymphocytic leukemia (CLL), six cases of mantle cell lymphoma (MCL), eight cases of follicular center lymphoma (FCL), eight cases of sporadic Burkitt's lymphoma (BL), nine cases of mucosa-associated lymphoid tissue type (MALT) lymphoma and eight cases of diffuse large B-cell lymphoma (DL-B). The diagnosis were established by using the criteria of REAL classification¹.

PCR amplification^{2,3}

A seminested PCR was performed in a GeneAmp PCR system 9600 (Perkin Elmer-Cetus). The first amplification was performed using a consensus VH primer (FR2A) and a JH primer (LJH). For each initial amplification, 1 µg DNA template was applied. For the reamplification, the LJH was replaced by a nested consensus JH primer (VLJH), and an aliquot (1 %) of the first PCR run was transferred as a template.

Cloning and DNA Sequencing^{2,3}

The DNA sequencing was performed on an automated DNA sequencer (Applied Biosystems, 373A) by using the DyeDeoxy Terminator method. The sequences obtained, were compared with published VH germ line sequences (GenBank). For the subcloning of the amplicates, the CloneAmp system (GIBCO BRL) was employed.

Results

The amplicates obtained were sequenced and investigated for homology with published VH germ line sequences. The results of this comparison (as shown in Table 1) reveal the presence of somatic mutations in all BLs, MALT lymphomas, FCLs and DL-Bs, whereas in all instances the B-CLLs and the MCLs expressed only VH germ line sequences. The mutation frequency of BL and MALT lymphoma proved to be different from that of FCLs and DL-Bs. As Table 1 shows, the average frequency of nucleotide exchanges was in BL and MALT lymphoma, at 4.9% and 6.5%, respectively, only half that seen in FCLs (11.8%) and DL-Bs (13.9%). Subcloned DNA from IgH rearrangements revealed identical VH sequences within all three cases of BL, the three cases of MALT lymphoma and the three cases of DL-B studied, indicating that the mutation process was switched off. In contrast, the clones obtained from the VDJ amplicates of FCLs showed nucleotides exchanges within all eight cases, which demonstrates ongoing mutation in all instances. A comparison of the VH mutation frequency with those of normal B cells at different stages of differentiation revealed a resemblance of B-CLL and MCL to sIgM+ IgD+ blood B cells and mantle cells, respectively, of FCL to later stage GC cells, and of BL and MALT lymphoma to the sIgM+ IgD-memory B cells (Fig. 1 and Table 2)⁴.

Table 1. Patterns of somatic mutations of the VH region genes in the various B cell malignancies

	Number of studied cases	Number of cases with IgH-R VH mutation	Number of somatic mutation	Mutation	
				Average frequency	ongoing (range)
B-CLL	6	0	0	0%	no
MCL	6	0 (1)	1	0 % (0-1)	no
Burkitt's	8	8	57	4.9 % (1-16)	no
MALT	9	9	86	6.5 % (1-16)	no
FCL	8	8	138	11.8 % (9-31)	yes
DL-B	8	8	162	13.9 % (11-38)	no

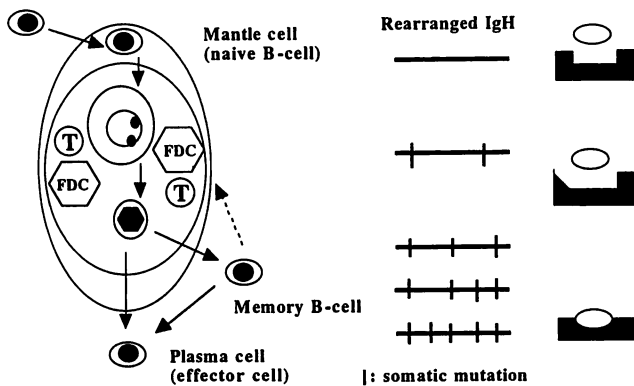


Figure 1. Somatic mutation of IgH gene & B-cell differentiation

Conclusions

The sequence analysis of the VH region genes involved the VDJ rearrangement from cell at different maturation stages of the B cell immune response has revealed that the increase in the affinity of the antibody-binding site is caused by hypermutation in the rearranged V region genes. The GCs could be identified as the site where the hypermutation process takes place. In a recent study^{5,6}, the development of V gene mutation in GC was analyzed in more detail by single cell analyses from different zones of the GC. This study revealed that, 1) GCs are dominated by a few large B cell clones exhibiting intraclonal diversity by ongoing mutation; 2) the mutation process starts in the dark zone of GC because the blasts in this zone, designated centroblasts, contain V genes with no or only a few mutations; 3) the mutation process proceeds when the centroblasts migrate to the light zone and transform into centrocytes because this was associated with an increase in VH mutations. The hypermutation and differentiation process may be associated with isotype switch. When leaving the GC, centrocytes differentiate into sIgM+ IgD- or sIgM-IgD- (ie, IgG- or IgA-expressing) memory cells, or into sIg- cytoplasmic Ig+ plasma cells. The sequence analysis of the rearranged Ig genes of peripheral blood B cells revealed mutated Ig genes in the memory cells, whereby the mutation frequency was twice as high in sIgM- IgD-memory B cells as in sIgM+ IgD- memory B cells in contrast to the sIgM+ IgD+ naive B cells, which lacked mutations in their rearranged Ig V genes. In both types of memory cells, there were no signs of ongoing mutations in contrast to the GC B cells. Thus, sIgM+ IgD- and sIgM-IgD- memory B cells appear to represent GC-derived B cells that had acquired their mutations within the GC. The sIgM+ IgD- cells may have left the GC at an early stage without undergoing isotype switching. Therefore, we could suppose the normal counterpart of various B-cell malignancies, that B-CLL and MCL originate from pre-GC naive B-cells, BL and MALT lymphoma originate from memory B-cells without class-switch and FCL originate from GC B-cells.

In contrast our results, Bahler et al.⁷ and Chapman et al.⁸ have reported that there are ongoing Ig gene mutations in salivary gland primed MALT lymphoma and sporadic BL, respectively. We are now investigated the reason why our studies could not detect the ongoing mutations in those lymphomas.

Table 2. Relation of the immunoglobulin V gene mutation pattern to B cell differentiation and maturation

Cell typ/ Differentiation stage	sIg	Mutation	
		Average frequency	Ongoing
Pre-GC compartment			
Pre-B cell	-	none	no
Immature B cell	IgM high	none	no
GC compartment			
Peripheral blood naive B cell	IgM & IgD high	none	no
Follicle mantle cell	IgM & IgD high	none	no
Post-GC compartment			
Dark zone			
Centriblast	- or IgM low	low to medium	yes
Light zone			
Centrocyte	IgM or IgG or IgA or -	medium to high	yes
Post-GC compartment			
Memory B cell without isotype switch			
Memory B cell without isotype switch	IgM high	low to medium	no
Memory B cell with isotype switch			
Memory B cell with isotype switch	IgG or IgA	medium to high	no
Plasma (effector) cell	- (cIg)	medium to high	no

Reference

- Harris, N.L., Jaffe, E.S., Stein, H., Banks, P.M., Chan, J., Cleary, M.L., Delsol, G., De Wolf-Peeters, C., Falini, B., Gatter, K., Grogan, T.M., Isaacson, P.G., Knowles, D.M., Mason, D.Y., Muller-Hermelink, H.K., Pileri, S., Piris, M.A., Ralfkiaer, E., Warnke, R.. A Revised European-American Classification of Lymphoid Neoplasms: A proposal from the International Lymphoma Study Group. *Blood*, 1994 84,1361-1392.
- Hummel, M., Tamaru, J., Kalvelage, B., Stein, H. Mantle cell (previously centrocytic) lymphomas express VH genes with no or very little somatic mutations like the physiological cells of the follicle mantle. *Blood*, 1994 84, 403-407.
- Tamaru, J., Hummel, M., Zemlin, M., Kalvelage, B., Stein, H. Hodgkin's disease with a B cell phenotype often shows a VDJ rearrangement and somatic mutations in the VH genes. *Blood*, 1994 84, 708-715.
- Tamaru, J., Hummel, M., Marafioti, T., Kalvelage, B., Leoncini, L., Minacci, C., Tosi, P., Wright, D., Stein, H. Burkitt's lymphomas express VH genes with a moderate number of antigen-selected somatic mutations. *Am. J. Pathol*, 1995 147, 1398-1407.
- Kueppers, R., Zhao, M., Hansmann, M-L., Rajewsky, K. Tracing B cell development in human germinal centers by molecular analysis of single cell picked from histologic sections. *EMBO J*, 1993 12, 4955-4967.
- Klein, U., Kuppers, R., Rajewsky, K. Human IgM+ IgD+ B cells, the major B cell subset in the peripheral blood, express V β genes with no or little somatic mutations throughout life. *Eur. J. Immunol*, 1993 23, 3272-3277.
- Bahler, D.W., Miklos, J.A., Swerdlow, S.H. Ongoing Ig Gene Hypermutation in Salivary Gland Mucosa-Associated Lymphoid Tissue-Type Lymphomas. *Blood* 1997 89: 3335-3344.
- Chapman, C.J., Zhou, J.X., Gregory, C., Rickinson, A.B., Stevenson, F.K. VH and VL gene analysis in sporadic Burkitt's lymphoma shows somatic hypermutation, intraclonal heterogeneity, and a role for antigen selection. *Blood* 1996 88: 3562-3568.