Isotype-switched immunoglobulin genes with a high load of somatic hypermutation and lack of ongoing mutational activity are prevalent in mediastinal B-cell lymphoma

Frank Leithäuser, Martin Bäuerle, Minh Quang Huynh, and Peter Möller

Primary mediastinal B-cell lymphoma (PMBL) is a subentity of diffuse large B-cell lymphoma with characteristic clinical, histomorphologic, immunophenotypical, and genetic features. Unlike other B-cell lymphomas, PMBL has not yet been the subject of comprehensive molecular studies on the rearranged immunoglobulin (Ig) gene. Such investigations have proved essential to obtaining information about the differentiation stage of the lymphomagenic B cell. In the present study, the clonally rearranged immunoglobulin heavy-chain gene of 13 PMBL cases is analyzed by polymerase chain reaction (PCR) in conjunction with cloning and DNA sequencing. Twelve of 13 rearrangements were potentially functional. All clonally rearranged immunoglobulin genes bore a high load of somatic mutations (average, 13.0%), which appeared to be selected for a functional antibody in the majority of cases. The comparison of cloned PCR products revealed no evidence of ongoing mutation of the immunoglobulin variable gene. By means of reverse-transcriptase PCR, lymphoma-specific immunoglobulin transcripts were detected in 8 of 13 cases, all of which were of the postswitched type, whereas immunoglobulin protein expression was undetectable except for 1 case. A PMBL cell line, MedB-1, generated from an IgG- parental tumor, constitutively expressed IgG protein in a subset of cells, which was moderately suppressed by interleukin-4 and up-regulated in the presence of dexamethasone. PMBL is thus characterized by a heavily mutated, class-switched immunoglobulin gene without evidence of ongoing mutational activity. Moreover, our data indirectly suggest that regulation by extrinsic signals contributes to the immunoglobulin-negative phenotype of PMBL. (Blood. 2001;98: 2762-2770)

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Patients, materials, and methods

Patients

Twenty patients with a diagnosis of PMBL were randomly selected on the basis of the availability of frozen material. Of these patients, those cases with detectable clonal IgVH gene rearrangements were further investigated in the present study. Clinical data of these patients are summarized in Table

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1. The age at diagnosis ranged from 21 to 59 years (mean, 38 years). Six patients were female, and 7 were male. The stage of disease was between 2 and 4.

**Tissue and cell line**

One tissue sample was taken from lymphoma invading the lung of a patient who had developed recurrent disease after receiving antitumor chemotherapy (case 8a). A stable cell line previously generated from this tumor (MedB-1, designated case 8b) was also included in this study. From one patient, 2 sequential samples were available. The first was taken at lymphoma diagnosis from a tumor mass located in the anterior mediastinum (case 13a), and the second was taken 1 year later at autopsy following chemotherapy and lymphoma progression to extensive disease (case 13b). All other specimens derived from primary tumors localized in the mediastinum without preceding chemotherapy. The diagnosis of PMBL was made according to established clinical, histomorphologic, and immunophenotypic criteria. Histologically, all cases exhibited a diffuse growth pattern and a variable degree of sclerosis. Tumor cells were of moderate to large size with abundant and often pale-staining cytoplasm and irregularly shaped nuclei lacking prominent nucleoli. The immunophenotype was consistently with abundant and often pale-staining cytoplasm and irregularly shaped

**DNA extraction**

Frozen tissue was cut into 25-μm slices on a cryostat microtome; a clean blade was used for every new tissue sample. Five tissue sections per case or pellets of 107 MedB-1 cells or peripheral blood leukocytes from healthy donors as polyclonal control samples were digested with 100 μg/mL Proteinase K (Sigma-Aldrich, Deisenhofen, Germany) at 50°C for 16 hours in a lysis buffer (10 mM Tris-HCl, pH 7.6; 10 mM EDTA; 50 mM NaCl; 0.5% sodium dodecyl sulfate [SDS]). After the addition of 4 volumes of a saturated NaCl solution and centrifugation at 10,000g, DNA was ethanol-purified from the supernatant and washed twice in 70% ethanol.

**Polymerase chain reaction**

We submitted 500 ng DNA to a polymerase chain reaction (PCR). Amplification of the VDJ rearrangement of the IgVH gene was performed with 2 separate primer sets. The first primer set (designated frame region 1 [FR1]) encompassed a consensus primer (FR1c) of FR1,20 in conjunction with a JH primer mix 21 containing primers JH 6a and JH 1245, with a JH consensus primer (VLJH) located internally to LJH. The second amplification round was carried out for 25 cycles at 60°C. Then 10 μL PCR product was size-fractionated on a 2% agarose gel and stained with ethidium bromide. All PCR analyses were performed at least in duplicate.

**Cloning and sequencing of PCR products**

PCR products were size-separated on a 2% agarose gel and specific bands excised from the gel. DNA was isolated and purified by gel chromatography (QiAqquik gel extraction kit) (catalog No. 28104; Qiagen, Hilden, Germany); ligated into a PCR-cloning vector (pGEM-T-Vector-System) (catalog No. A3610; Promega, Mannheim, Germany) at a molar insert-to-vector ratio of 3:1; and subsequently transformed into competent JM109 bacteria. Bacteria were plated on Luria Bertani (LB)—agar plates containing ampicillin, X-Gal (Sigma-Aldrich), and isopropylthiogalactoside (Sigma-Aldrich) at concentrations recommended by the manufacturer, and were grown overnight at 37°C. White colonies were picked, and the presence of an insert was confirmed by restriction enzyme analysis with SacI and ApaI. Plasmid DNA was prepared according to an established alkaline lysis protocol. Sequencing was always performed on both strands with an Alphal Express Sequencer (Amersham Pharmacia Biotech) by means of the Auto Cycle Kit (catalog No. 27-2693-02; Amersham Pharmacia Biotech) as recommended by the manufacturer.

**Analysis of sequencing data**

Sequences of clonal VDJ rearrangements were compared with published germline sequences of the human IgVH gene by means of DNAPLOT Version 2.0.1 and the V BASE Version 1.0 (available at: http://www.mrc-cpe.cam.ac.uk/mtm-doc/Restricted/ok.html. Accessed August 6, 2001). Case 3 did not yield an alignment by DNAPLOT owing to sequence deletions and was therefore analyzed by means of the IgBLAST software (available at: http://www.ncbi.nlm.nih.gov/igblast/. Accessed August 6, 2001). To determine whether mutations of the IgVH gene were random or selected for, the ratio between replacement and silent mutations (R/S ratio) of the framework regions was determined and compared with the R/S ratio to be expected if the mutations merely happened by chance. These calculations were carried out with the computer software Inh Sus Calc V 1.0 and R S Probability, kindly provided by Dr Paolo Casali and Dr Hong Zan (Division of Molecular Immunology, Department of Pathology, Cornell University Medical College, New York, NY). In codons with 2 or 3 substitutions, each mutation was classified separately as R or S in comparison with the germline sequence of the codon.

**Reverse-transcriptase PCR**

Ten slices of 25 μm each were cut off frozen tissue blocks, collected in a FastRNA Tube (catalog No. 6040-601; Bio 101, Carlsbad, CA), and mechanically homogenized in Trizol reagent (catalog No. 15596-026; Life Technologies, Karlsruhe, Germany) by a FastPrep 120 device (Bio 101). RNA was extracted according to the instructions by the manufacturer of the Trizol reagent. Then, 5 μg RNA was annealed to 1 pmol oligo(dT)12 primer, denatured at 80°C, and reverse transcribed with 200 U Super Script reverse transcriptase (catalog No. 18053-017; Life Technologies) in 25 μL reaction mix containing buffer supplied by the manufacturer, 10 mM dithiothreitol, 40 U ribonuclease inhibitor (RNasin) (catalog No. N2111; Promega), and 1 mM each dNTP. The reverse transcription was carried out at incremental temperature steps ranging from 25°C to 95°C. We submitted 1 μL reverse-transcriptase (RT) reaction product to a PCR analogous to the above...
We incubated 5 \times 10^6 MedB-1 cells with 100 U/mL interleukin-4 (IL-4) (catalog No. 50444; Biomol, Hamburg, Germany) or 1 to 100 \mu g/mL dexamethasone (catalog No. D2915; Sigma-Aldrich) in RPMI 1640 medium (catalog No. 21875, Life Technologies) to which 100 IU/mL penicillin (catalog No. 15140-141; Life Technologies), 100 \mu g/mL streptomycin (catalog No. 15140-114; Life Technologies), and 2 mM L-glutamine (catalog No. 21051-016; Life Technologies) had been added. After stimulation for 48 hours, cells were washed twice in PBS buffer. Then, 2 \times 10^6 cells were resuspended in a lysis buffer (50 mM Tris-HCl, pH 7.5; 150 mM NaCl; 1% Nonidet P-40; 0.5% sodium deoxycholate) containing 1 tablet of Complete protease inhibitor cocktail (catalog No. 1697498; Boehringer Mannheim) per 50 mL buffer and mechanically disrupted on ice in a dounce homogenizer with the use of a 2 mL polished tube with an appropriate Teflon pestle. Cellular debris was removed by centrifugation at 13 000 \times g for 5 minutes at 4°C. The supernatant was transferred to a fresh tube and the protein concentration measured according to the method described by Lowry et al.\textsuperscript{24}

**Southern blot**

Southern blotting of the RT-PCR products was carried out according to established protocols. Briefly, agarose gels were incubated in 0.5 M NaOH and 1.5 M NaCl to denature the DNA, followed by neutralization in 0.5 M Tris buffer (pH 7.5) and 3 M NaCl. PCR products were transferred to a Hybond N\textsuperscript+ membrane (catalog No. RPN1520B; Amersham Pharmacia Biotech) by a conventional capillary blot with 10 \times SSC as a transfer buffer and were subsequently cross-linked to the membrane by baking for 2 hours at 80°C. Specific PCR products were detected via hybridization to \textsuperscript{32}P-labeled isotype-specific oligonucleotide probes (\mu g/mL GGT CAT CCG CCC CCA CC; \gamma, CAC CCA CAA CTC CGG AT; \alpha, GAG GGC CCA CCA TCG GTC TTC CC; \kappa, CAT CCC CGA GCC CCA CCA AG; e, CCT CCA CAC AGA GCC CAT CC; \mu, TTC TCC TTG ACC AGG CAG CC; \delta, GTC ACG GAG GTG GCA TTG GA). Amplification was carried out at 95°C for 16 hours at 68°C in 5 \times SSC containing 0.1% N-lauroyl-sarcosine, 0.02% SDS, 2% blocking reagent (catalog No. 1175041; Boehringer Mannheim), and 5 pmol/mL labeled probe. After 2 posthybridization washes in 2 \times SSC/0.1% SDS at room temperature for 15 minutes, bound probe was detected by antidigoxigenin Fab fragments and chemiluminescent detection with CSPD by means of the DIG Luminescent Detection Kit (catalog No. 1363514; Boehringer Mannheim) according to the manufacturer’s instructions. Chemiluminescence was visualized by exposing the membranes to x-ray film for 15 to 120 minutes at room temperature.

**Enzyme-linked immunosorbent assay**

To quantify IgG in stimulated MedB-1 cells, an IgG enzyme-linked immunosorbent assay (ELISA) kit (catalog No. K 6510; Cell Concepts, Umkirch, Germany) was used according to the manufacturer’s instructions. Cell lysates containing 100 \mu g protein were added to the wells of a microtiter plate precoated with a polyclonal anti-human IgG rabbit antibody. After washing, a peroxidase-conjugated polyclonal anti-human IgG rabbit antibody was added. Bound antibody was detected by the addition of tetramethylbenzidine substrate and measurement of the extinction in an MRX ELISA-reader (Dynatech Laboratories, Denkendorf, Germany) at 450 nm.

**Results**

**Detection of clonal VDJ rearrangements**

Clonal IgV<sub>H</sub> gene rearrangements were amplified from 13 of 20 cases (65%) (Figure 1). Eight cases yielded clonal signals with primer combination FR1. Five cases were negative with this primer set but showed clonal PCR bands after analysis with the alternative

![Figure 1. Detection of clonal VDJ rearrangements in PMBL. DNA from PMBL tissue was submitted to a PCR with primer combination FR1. Cases that did not reveal clonal signals with FR1 were reanalyzed with an alternative primer set FR2. Monoclonal rearrangements are identified by a sharp, distinct band in comparison with diffuse signals generated from polyclonal control samples (P). In cases 8a, 8b, and 11, specific PCR products are indicated by an arrowhead. Additional bands detectable in these cases proved to be nonspecific upon sequence analysis. M indicates molecular size marker.](www.bloodjournal.org)
cases 8b and 13b.

DLBCL when VH gene family–specific primers were applied. clonal VDJ rearrangements could be amplified from 40% of mutations introduced in a random fashion. As pointed out by Klein significantly lower than the R/S to be expected in the case of antibody, we calculated whether the detected R/S ratios were to have been introduced during the process of somatic hypermutation, impairing the hybridization of the primers. In fact, a rate of 30% to 50% of false-negative results is commonly found in analysis of VDJ rearrangement by PCR with the use of consensus primers. In a recent study, no clonal VDJ rearrangements could be amplified from 40% of DLBCL when VH gene family–specific primers were applied.

Rate of somatic mutation

The results of the DNA sequence analysis are summarized in Table 2. All clonal rearrangements were monoclonal; additional bands amplified from cases 8a, 8b, and 11 proved to be nonspecific upon sequence analysis. Seven specimens (35%) generated polyclonal amplification products from which dominant clonal bands were absent. Undetected monoclonality in PCR assays of the VDJ rearrangement can be attributed to alterations of the target DNA by somatic hypermutation, impairing the hybridization of the primers. In fact, a rate of 30% to 50% of false-negative results is commonly found in analysis of VDJ rearrangement by PCR with the use of consensus primers. In a recent study, no clonal VDJ rearrangements could be amplified from 40% of DLBCL when VH gene family–specific primers were applied.

Distribution of mutations

To determine whether mutations were selected for a functional antibody, we calculated whether the detected R/S ratios were significantly lower than the R/S to be expected in the case of mutations introduced in a random fashion. As pointed out by Klein et al, mutational patterns within the CDR are difficult to interpret. Depending on the affected amino acid, some R mutations in the CDR may have little effect on the antibody affinity while others occurring within an already favorably mutated IgVH gene could dramatically reduce the binding affinity and thus even be counterselected. Therefore, we concentrated on the analysis of the FR and did not consider the CDR. In 12 cases, the VDJ rearrangements were in frame and potentially functional. The only nonfunctional VDJ rearrangement was found in case 3, which was out of frame and displayed 2 stop codons. In 10 of the 12 cases (83%) with potentially functional rearrangements, replacement mutations within the frame regions occurred significantly less frequently (P < .05) than would be expected by chance. Moreover, the mean value of observed R/S ratios was 1.4 (range, 0.7 to 3.4) and therefore within the range of magnitude found in normal memory B cells and plasma cells that can be assumed to have undergone affinity selection. These results strongly suggest that antibody-mediated selective forces maintained the overall structure of the B-cell receptor in PMBL.

Ongoing mutation

To ensure that base exchanges were not introduced by errors of the Taq polymerase (Taq error in our laboratory, 0.13%), mutations were only counted as such if they were present in at least 2 clones.

### Table 2. Analysis of VH gene sequences

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Primer combination</th>
<th>Corresponding VH germline</th>
<th>In frame</th>
<th>Percentage mutation</th>
<th>Observed mutations in FR</th>
<th>R/S</th>
<th>Observed</th>
<th>Expected</th>
<th>P value</th>
<th>No. clones sequenced</th>
<th>Ongoing mutation</th>
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<td>NA‡</td>
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<td>NA‡</td>
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Sequences have been submitted to the EMBL/GenBank/DDBJ database under accession numbers AJ292059 through AJ292070. See Table 1 footnote for descriptions of cases 8b and 13b.

FR indicates frame region; R, replacement mutation; S, silent mutation; NA, not analyzed.

*P < .05.

†No intraclonal diversity was detectable, but this was of limited significance owing to low number of sequenced clones.

‡Not analyzed because of nonfunctional rearrangement.
IgG was observed 3 times. IgG was a
IgM was observed twice and IgE shown). IgG PCR bands and hybridization signals, respectively (data not
found. This was not due to low sensitivity of the RT-PCR or
use of sequence analysis, could be unambiguously assigned to the
Corresponding lymphoma cell clone. No IgG
immunoglobulin isotypes by RT-PCR with lymphoma-specific
isotype-specific probe, PCR products yielding positive signals
were detected in 5 cases, among which VH1 could also be detected twice (16.7%), and VH2 and VH5 were each
found once (8.3%). Thus, no bias toward use of the VH4 family was
obvious in comparison with normal peripheral B cells.22

Expression of isotype mRNA

We amplified IgH mRNA composing the 3’ part of the variable
chain and the 5’ end of the first exon of the constant chain of all 5
immunoglobulin isotypes by RT-PCR with lymphoma-specific
primers. Following Southern blotting and hybridization to an
isotype-specific probe, PCR products yielding positive signals
(Figure 4A) were cloned and sequenced (Table 3). In 8 cases
(61.5%), immunoglobulin transcripts were detected that, with the
use of sequence analysis, could be unambiguously assigned to the
corresponding lymphoma cell clone. No IgA or IgE mRNA was
found. This was not due to low sensitivity of the RT-PCR or
Southern blot assay, since positive control samples gave strong
PCR bands and hybridization signals, respectively (data not
shown). IgY transcripts were detected in 5 cases, among which
IgY1 was observed twice and IgY2 was observed 3 times. IgA was
detectable in 2 cases, and 2 cases were shown to express IgE. A
simultaneous expression of IgY2 and IgE mRNA was observed in
case 4. The coexpression of constant region transcripts from 2
different isotypes by one lymphoma has been previously described
in follicular lymphoma and DLBCL.33 Possible mechanisms ac-
counting for this phenomenon are differential deletional switch
recombination in subpopulations of the lymphoma or switching
without DNA recombination by alternative splicing or transsplic-
ing.34 In case 3, IgA was not directly connected to VH3DJH6C but
joined 373 bp downstream into the intron adjacent to JH6C. The
intronic sequence displayed a high level of somatic hypermutation,
which was more pronounced in the 5’ half (13.9%) than in the 3’
half (8.7%). No mutations were found in the Cα exon 1 covered
by the sequence analysis. As illustrated in Figure 4B, somatic mutation
generated a T→G transversion at position 2 of the intron that
led to a destruction of the regular donor splice site and use of a
cryptic donor splice sequence at position 373 (AG277GTGCGC)
(Senapathy score35 of the cryptic donor site: 79.4).

Immunohistochecmy

All cases were intensely CD20+ on immunohistology (Table 3).
J-chain expression was detectable in subpopulations of neoplastic
B cells in 2 cases (cases 2 and 13a) and negative in all other cases.
In one case (13a), a coexpression of IgA and J chain was observed.
In this case, the expression of IgA/κ protein correlated with the
detection of IgG transcripts. It is of note that lymphoma tissue taken
at autopsy of the same patient 1 year later was negative for IgA/κ
and J chain but still expressed IgG transcripts (Table 3; Figure 4A).
In no other case was there an expression of light-chain or
heavy-chain isotypes.

Stimulation of the MedB-1 cell line

In contrast to the IgG− parental lymphoma tissue of case 8 (Figure
5A), the MedB-1 cell line derived from it was found to express
IgG/κ protein in the cytoplasm of a small fraction composing fewer
than 10% of the cells (Figure 5B; Table 3). IgG expression was
moderately influenced upon addition of stimulating substances.

Figure 3. Evidence for ongoing mutation in MedB-1 cells. Seven PCR clones
amplified from MedB-1 cells were aligned and compared with the most homologous
germine IgVH sequence V4-39 from codons 86 through 92. Sequence homologies
between V4-39 and the consensus sequence of the PCR clones are highlighted by
asterisks. Dashes designate identities among PCR clones. Clones 3 and 6 show
deviations from the consensus sequence in codons 87 and 91, suggesting ongoing
somatic mutation.

Use of VH families

In 6 of 12 potentially functional VDJ sequences (50.0%), members of the
VH3 family were rearranged. VH4 was found in 2 cases
(16.7%), of which VH-43 was amplified from one sample. VH1
could also be detected twice (16.7%), and VH2 and VH5 were each
found once (8.3%). Thus, no bias toward use of the VH4 family was
obvious in comparison with normal peripheral B cells.22

Figure 4. Detection of VHH-CCH transcripts of the IgH gene. VH-CCH transcripts were
amplified by RT-PCR with lymphoma-specific 5’ primers and isotype-specific 3’
primers, transferred to nylon membranes, and labeled by isotype-specific probes (A).
The identity of the PCR products was confirmed by cloning and sequencing. In case
9, the expression of IgA/κ mRNA was observed. Sequence analysis of this
transcript was observed. Sequence analysis of this transcript (B) revealed an aberrant
VH-CCH splicing, caused by a T→G transversion within the regular donor splice site at the 3’
end of VH-DJH6C and Cα exon 1 (both indicated by gray background) are
separated by a 373-bp intronic sequence. Note the high rate of somatic hypermuta-
tion carried far into the intron. This sequence has been submitted to the EMBL/
GenBank/DDBJ database under accession number AJ292071.
Whereas IL-4 (100 U/mL) led to a down-regulation by 32% (Figure 5C), there was a 1.8-fold increase of IgG expression in the presence of 10 μg/mL dexamethasone (Figure 5D) when compared with nonstimulated cells. Immunocytoology confirmed the ELISA results (Figure 5F) and demonstrated that the modulatory effect was restricted largely to the constitutively IgG^1 cell fraction while the number of IgG-expressing cells remained essentially unaltered. No IgG was found in the epithelial control cell line MiaPaca (Figure 5E).

### Discussion

The PMBL analyzed in the present study carried a high load of somatic mutation. The mean base exchange rate of 13.0% in our lymphoma panel exceeds the mutation level found in normal germinal center and memory B-cell populations\(^36\) and is slightly higher than the average mutation frequency of DLBCL, ranging from 8.5%\(^37\) to 9.9%.\(^13\) This raises the question as to whether ongoing mutation within the neoplastic clone contributed to the high mutational load in PMBL as has been demonstrated for follicular center lymphoma.\(^38\) Applying stringent criteria for the definition of intraclass diversity,\(^28\) we found no differences among the individual PMBL cases. Moreover, identical VDJ sequences were amplified from a biopsy sample and from lymphoma tissue taken 1 year later at autopsy of the same patient (cases 13a and 13b). Reports about ongoing mutation in DLBCL are inconsistent; while some groups demonstrated ongoing mutation in DLBCL,\(^33,39\) others did not.\(^37,40\) Data published by Ottensmeier et al.\(^33\) demonstrate that anti-tumor chemotherapy or disease progression is related to a loss of intraclass variation in DLBCL that had initially displayed ongoing mutation at primary diagnosis. However, this mechanism does not explain the absence of ongoing mutation in the

<table>
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See Table 1 footnote for descriptions of cases 8b and 13b. − indicates no positive cells; +/−, 50% positive cells; +/<−, fewer than 10% positive cells; +/<−, fewer than 50% positive cells; NA, not analyzed.

Figure 5. IgG protein expression of MedB-1 cells. In comparison with the IgG− parental tumor (A), a small subset of MedB-1 cells showed an expression of IgG protein, detectable by immunocytochemistry (B) and ELISA (F). Incubation for 48 hours in the presence of 100 U/mL IL-4 resulted in a down-regulation of IgG (C, F) whereas 10 μg/mL dexamethasone induced a moderate increase (D, F). ELISA data are given as mean value (n = 5) and SD. Panel E shows negative control pancreatic carcinoma cell line MiaPaca. Original magnification ×50 (A), and ×100 (B-E).
The finding of switched isotypes further differentiates PMBL from DLBCL, as the detection of switched immunoglobulin transcripts within the germinal center after somatic hypermutation has been triggered. The rearrangement of IgVH gene to conserve the antibody structure and maintain the potential for a functional B-cell receptor. Evidence for selection of a functional antibody in a large subset of cells. Interestingly, we also found a low degree of intrachromosomal variation in MedB-1 cells (Figure 3), whereas there was no evidence for ongoing mutation in the parental tumor. It remains to be clarified whether the reactivation of the mutational machinery is limited to the IgG- cell subset. Moreover, IgG expression could, albeit to a moderate extent, be downregulated in vitro by IL-4 (which also has the propensity to inhibit immunoglobulin production in normal human B cells35) and increased by dexamethasone, in accordance with the inductive effect of corticosteroid hormones on immunoglobulin expression at the late stage of human B-cell differentiation35 (Figure 5). We therefore provide suggestive evidence that immunoglobulin protein expression in PMBL is reversibly down-regulated in vivo by extrinsic signals, one of which might be IL-4.

Considering the presumably thymic origin of PMBL, it is a plausible and attractive hypothesis that this form of lymphoma originates from a specific subpopulation of B cells resident in the thymic medulla.3 Thymic B cells display a CD20+CD21+ IgM+ but IgG− immunophenotype.36 To date, the molecular analysis of the VDJ rearrangement of human thymic B cells has been the subject of only one study,37 which demonstrated that most rearrangements were in the germline configuration and that fewer than 20% showed a moderate rate of somatic hypermutation. Hence, PMBL diverges from its putative normal progenitor cell by means of the prevalence of class switching, the high degree of somatic hypermutation, and the lack of immunoglobulin protein expression. While immunoglobulin expression has probably been down-regulated after neoplastic transformation, it is unlikely that isotype switching
and somatic hypermutation of the IgV{\textgamma} gene occurred after neoplastic transformation had been fully completed. With the data presented in this study, a more precise identification of the thymic B-cell subset that gives rise to PMBL appears to be warranted. The lack of ongoing mutation and the prevalence of class switching further emphasize the distinct and rather homogeneous character of PMBL, providing additional justification for separating this lymphoma from other DLBCLs.

Acknowledgments

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References


Isotype-switched immunoglobulin genes with a high load of somatic hypermutation and lack of ongoing mutational activity are prevalent in mediastinal B-cell lymphoma

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